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THE STUDY OF INFLUENZA

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PREFACE

In recent years influenza has been studied very actively in the USSR and abroad. The problem is being worked out in the most varied directions, and there is not a single division of it, I daresay, in which essential theoretical or practical results have not been obtained.

The most definite achievement of recent years has been the obtaining of a live prophylactic vaccine therapeutic serum; putting these into mass production has given a reliable basis to the matter of controlling influenza.

There are no works either in the Soviet or foreign literature which generalize on the numerous investigations of recent years in the main divisions of the influenza problem. Monographs which have been previously published have either become outdated or include only individual divisions of the study of influenza. The book now being brought to the attention of readers is an attempt to fill this gap.

In the book, in addition to extensive bibliographic material (more than 2000 bibliographic sources), a generalization is given of the results of investigations carried on for the past 10-12 years by three groups of scientific workers under the direction of the authors of this book: The Institute of Virology imeni Ivanovskiy of the Academy of Medical Sciences USSR (V. M. Zhdanov, V. D. Solov'yev, F. G. Epshteyn), Khar'kov Institute of Vaccines and Sera imeni Mechnikov (V. M. Zhdanov) and The Moscow Institute of Vaccines and Sera imeni Mechnikov (V. D. Solov'yev). The authors have limited themselves to a presentation of the influenza problem only, without, as a rule, dealing with common colds in order to avoid distracting attention and increasing the size of the book, which is considerable even without this.

Chapters 1-5 have been written by V. M. Zhdanov; chapters 7 and 10, by V. D. Solov'yev; chapters 6, 8 and 9, by F. G. Epshteyn (the last two in collaboration with V. M. Zhdanov). Various sections of the book have been written by A. S. Gorbunova (section 14, 26, 27, 28, 41), L. L. Fadeyeva (sections 15, 44) and L. Ya. Zakstel'skaya (section 17). The authors would like to express their ap-

preciation to L. L. Fadeyeva for her aid in the technical formulation of the book.

The authors will be grateful for any criticism directed at eliminating defects in the book.

V. Zhdanov
V. Solov'yev
F. Epshteyn

FIRST CHAPTER

BRIEF HISTORICAL DATA

1. Definition

Influenza is an acute infectious disease of viral etiology, which chiefly involves the respiratory organs, is transmitted by the air-droplet route and not uncommonly assumes an epidemic or pandemic distribution.

The name "grippe" comes from the French word "gripper" -- to grasp. In a number of countries the other name of this disease -- influenza -- is used more frequently; it comes from the Latin word "influere" -- to invade, and according to another explanation, from the Italian term "influenza di freddo" -- the influence of cold. These names of the disease are at present generally accepted. Other names for it, namely, epidemic catarrhal fever, Spanish disease ("Spanish flu'") and others are no longer used.

The name "influenza" began to be used in the eighteenth century, although Makower (1953) found mention of the earlier use of this term (seventeenth century). He points out that the name "grippe," introduced by Sauvage in 1743 (la grippe), possibly comes from the Polish word "chrypy" or "chrypki," and he associates the occurrence of it in France with the stay of the Polish king Stanislau Leszczinski at Nancy in 1738; he also directs attention to the Czechoslovak name of grippe -- chripka. The Polish and the Czech names have a common Slavic root "khrip" (to speak hoarsely). At the present time, the name "influenza" is used in the Anglo-American literature; the name "grippe" is used in the USSR, the Slavic states, as well as in Germany and France.

It is not possible accurately to establish the time of origin of influenza. A description of diseases similar to influenza in their clinical course can be found even in Hippocrates.

They can hardly be considered to be influenza on the basis of a clinical similarity alone, however, with equal justification they may be looked upon as acute catarrhs or other similar diseases of the respiratory organs. The distinguishing feature of influenza is not only its

clinical course but also the characteristic features in its epidemiology: a sudden and precipitate spread of the disease, which in a short time embraces large population masses of the whole country, or several countries, or even of several continents. It is specifically this characteristic of the influenza epidemiology which has apparently served as the cause of the characteristic names of the disease (to grasp, to invade).

The ease of infection (droplet mechanism of transmission of the infective principle), the high degree of susceptibility, the short incubation period, the acute and brief course, accompanied by the elaboration of a strong, even if not lasting immunity, all are causes of the fact that influenza epidemics spread rapidly, including large masses of the population. However, these characteristics of influenza, which account for its extensive distribution in a developed society, make its existence improbable or even impossible in an old society in which there is insignificant socialization among people. It is not surprising, therefore, that influenza did not exist in America or Australia before their discovery by Europeans. For the same reasons influenza is not endemic on isolated islands, but rather appears in the form of epidemics, disappearing after they stop until it is reimported from without. It should be supposed, therefore, that influenza began as a new disease at a comparatively late stage of development of society, at a time when large governments were built up and communications among peoples became constant.

Actually, the first descriptions of epidemics of disease which can be more or less reliably considered influenza epidemics pertain to the beginning of the second thousand years A. D. According to Hirsch (1860, 1881), the first influenza epidemic, which included Italy, Germany and England, occurred in 1173; subsequent epidemics were described in the fourteenth century (1323, 1328, 1387), and the first pandemic, which included four continents (Asia, Africa, Europe and America), occurred in 1580. Francis evaluated the great epidemic of influenza in Edinboro in 1562 as a "new acquaintance" with this disease. In the fifteenth century, four large epidemics of influenza were described; in the sixteenth century, seven epidemics; in the seventeenth century, also seven; in the eighteenth century, 19 epidemics; and in the nineteenth century influenza became an endemic disease in the majority of the countries in the world, regularly producing large epidemics and pandemics. The pandemic of 1889-1890 was particularly widespread: during it, according to approximate calculations, 50 percent of the world's population had the disease. Among the numerous epidemics of the twentieth

century the pandemic of 1918-1920, which included almost the entire population of the globe and which killed about 20,000,000 persons (Gamaleya, 1922), has a special place.

Threfore, in the nineteenth century influenza became the most widespread infectious disease. At the presemt time, the influenza morbidity rate exceeds the morbidity rate for all other infectious diseases put together.

2. Study of the Etiology of Influenza

The development of microbiology at the end of the twentieth century created the basis for a scientific solution to the influenza problem. This problem began to be studied particularly actively in connection with the influenza pandemic of 1889-1890, at which time numerous observations were collected on the critical aspects and epidemiology of the disease.

Three periods may be distinguished in the study of the etiology of influenza: the period before 1918, at which time investigations were directed chiefly at a search for a bacterial pathogen; the period from 1918 through 1933, which terminated in the discovery of the influenza virus, and the subsequent period when the study of the entire influenza problem was put on a strong scientific foundation.

The study of the etiology of cases of influenza during the pandemic of 1889-1890 and in subsequent years was concluded with the discovery of a hemoglobinophilic bacterium by Afanas'yev (1891) and Pfeiffer (1892) -- the Bacillus influenzae Afanas'yev, Pfeiffer (Haemophilus influenzae), to which the role of causal organism of influenza was ascribed for a long time. The frequent finding of this microbe in the respiratory passages of patients with influenza and the presence of pathogenic properties in it served as the basis for this. Soon, however, the belief in the role of the Bacillus influenzae in the occurrence of influenza was shaken. Not uncommonly, the Bacillus influenzae was found in patients with other diseases and in healthy persons, and at the same time was absent from patients with influenza.

Thus, in 1918-1920, according to the extensive summarized data of Mote (1943), which includes several thousands of investigations, the Bacillus influenzae was isolated in 42 percent of the patients with influenza on the average. At the same time, in other diseases of the respiratory tract (diphtheria, measles, tuberculosis, pneumonia and others) it was isolated in 34 percent, and in 26 percent of healthy persons.

Experiments on animals made it possible to establish

the pathogenic properties of the Bacillus influenzae; however, it was not possible to reproduce the characteristic picture of influenza either in animals (including monkeys) or in people through the injection of this microbe into the respiratory passages. In this connection investigations on volunteers made by Smorodintsev and others (1936-1937) are illustrative: after the introduction of a suspension of the influenza bacilli into the respiratory passage mild signs of a cold developed, without the intoxication characteristic of influenza. At the present time, it has been shown that the Bacillus influenzae does not have any relationship to the etiology of influenza. Being a conditionally pathogenic microbe, it can become the cause of various diseases, chiefly of septic nature, as well as of secondary complications of various infections. In addition, there are grounds for identifying it with the Koch-Weeks bacillus, the pathogen of acute conjuctivitis (Pittman, 1945).

In the course of study of influenza ideas were expressed as to the etiologic role of bacteria. Specifically, Oritsky and Gates (1921) described a very small anaerobic bacillus as a possible causal organism of influenza -- Bacterium (Dialister) pneumosintes, and Pavlovic (1929) described a similar microbe, the Bacterium (Dialister) granuliformans. However, the etiologic significance of these bacteria in influenza was not confirmed. A large number of the most varied microbes has been described as causal agents of influenza in the Soviet Union and abroad during the end of the past century and the first quarter of the present century. It is hardly necessary to list these findings, because their etiologic role was not confirmed subsequently, and many of the microbes described were completely inadequately studied.

Observations made during the influenza pandemic of 1918-1920 made it possible for a number of authors to suggest the idea of the viral etiology of influenza. This idea, which was first expressed by Kuse, was confirmed by his student, Salter (1918), in experiments. Salter injected himself and an assistant with the filtrates of the sputum of influenza patients, as a result of which they became ill. Somewhat later in the same year, Nicolle and Lebailly (1919) produced the disease in two volunteers after the subcutaneous injection of sputum filtrates of patients. Diseases similar to influenza developed in monkeys after infection of them with sputum intranasally and into the conjunctivae of the lids. Positive results were also obtained in self-infection experiments by Dujarric de la Riviere (1942), in whose monograph a summary is presen-

ted of investigations on the detection of the influenza virus which were carried out in 1918-1919 in people and monkeys. However, these experiments were not confirmed by investigations for the pur pose of verification of them. Schmidt (1920) and others obtained doubtful data. Odyurua (1936) has given a summary of the investigations of this period.

In evaluating the negative results of these experiments, it should be noted that they could have been caused by unfortunate selection of volunteers immune to influenza or by the absence of the virus in the material being tested (Smorodintsev, 1957). Contradictory results in experiments on volunteers were the reason that the viral etiology of influenza remained unproved for a long time.

Shope's (1941) investigation on the etiology of swine influenza were of great importance in solving the problem of the etiology of influenza. This author showed that swine influenza is produced by a filterable virus, and that the disease has a more severe course if a secondary infection with the bacillus of swine influenza, similar to the bacillus of Afans'yev-Pfeiffer (Hemophilus influenzae suis), is superimposed on it.

Soon after the publication of Shope's works the virus of human influenza was discovered. During an influenza epidemic, Smith, Andrewes and Laidlaw (1933) infected various animals with washings from nasopharynx of patients which had been passed through bacterial filters. All the animals proved to be resistant except for African ferrets, which became sick after the intranasal introduction of the material. The investigators succeeded in isolating the virus from five out of eight influenza patients, whereby a control introduction of washings from four healthy persons and one suffering with a common cold did not produce any disease phenomena in the ferrets. After a two-day incubation period the temperature in the infected animals increased (usually biphasically), catarrh of the respiratory passages appeared with the excretion of mucus from the nose. The disease was not lethal and was transmitted to healthy ferrets in contact with the sick animals or through the introduction of excretions into their noses taken from the respiratory passages of the sick animals. The infective principle passed through fine-pored filters. The serum of the animals which had had the disease neutralized the virus: after the introduction of the virus in a mixture with serum of people who had had influenza the animals did not become sick. The virus of swine influenza which had been obtained by the authors from Shope produced a similar disease picture in ferrets, and both viruses produced an incomplete crossed immunity to one another.

The discovery made by the English authors was soon confirmed by other research workers in various countries, including the USSR, where as early as 1936 Smorodintsev and his colleagues isolated and identified the influenza virus. It was soon established (Andrewes, Laidlaw, and Smith, 1934) that white mice are susceptible to influenza: after a number of successive intranasal passages the influenza virus produces a fatal pneumonia, and then the possibility was shown of isolating the virus from people sick with influenza by means of the intranasal infection of mice with nasopharyngeal washings (Zil'ber, Fal'kovich and Arkhina, 1937). For the purpose of further investigations the development of the method of influenza virus culture on chick embryos was important (Smith, 1935; Burnet, 1935; Barykhin, Shakhmaliyeva and Bauer, 1936).

Before 1940 it was believed that influenza is caused by a single causal agent. After the investigations of Francis (1940) and Magill (1940) it was made clear that two influenza pathogens exist which are distinguished in their antigenic structure and in certain biologic properties. The first of the viruses discovered was called the "A" virus; the second one, the "B" virus. Afterwards, two other pathogens were discovered -- the "C" virus (Taylor, 1949) and the "D" virus (Kuroya, Ishida, Shiratori), as well as the "A-prime" (Rasmussen, Stokes, Smadel, 1948), which is now considered to be a variety of the "A" virus. In investigations of recent years (Smorodintsev and Luzyanina, 1948, 1950, 1953; Zhdanov and Solov'yev, 1951; Zhdanov, 1953; Gorbunova, 1953) the most important rules and regulations of variability of influenza viruses were studied in the epidemic process, and a classification of influenza viruses was worked out (Gorbunova, 1953). Investigations on the variability of influenza viruses were particularly extensively developed. On the basis of these investigations certain principal problems of influenza epidemiology were revised, and the basis of specific prophylaxis of it was worked out.

The establishment of the viral etiology of influenza and the study of its causal organism made it possible to separate out two groups of diseases from the total mass of diseases recorded as "influenza": virus influenza and acute (seasonal) catarrhs of the respiratory passages (Smorodintsev, 1938, 1939, 1949, 1950). This classification of influenzal diseases obtained prompt recognition in the Soviet Union, and differential diagnostic tables were worked out on the basis of it which are extensively applied in the work of therapeutic-diagnostic institutions. One of the latest publications of this Table (1951), supplemented by data on the etiology of catarrhs of the upper respiratory

tracts (Table 1), is presented below.

Table 1

Table for the Differential Diagnosis of Influenza and Acute Catarrhs of the Upper Respiratory Tracts Worked Out by the Committee for Influenza Control of the Ministry of Health USSR

Characteristics	Influenza	Acute Catarrhs of the Upper Respiratory Tracts
1	2	3
	Etiologic Data	
Pathogen	Filtrable virus types "A," "B," "C," and "D".	Frequently, pneumococci and other microbes as well as recently discovered adenoviruses and other agents of viral origin are of importance in the development of the disease.
	Epidemiologic Data	
Nature of distribution	Occurrence of epidemics does not always depend on the season of the year; the epidemics spread rapidly.	A connection has been noted with changes in the external temperature; spread is observed most often in the transitional parts of the year -- in the spring or the autumn.
Contagiousness	Considerable	Usually not very contagious in adults; not uncommonly considerably contagious in children.
Localization	Frequently observed	Observed less often.
Catarrhal factor	Not of decisive importance	Is of importance.
	Clinical Data	
Onset of the disease	Sudden, usually with a chill, most often without a prodromal period.	More often gradual.
Signs of intoxication	Marked for the most part.	Absent in the majority of cases

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Table 1 [continued]

Temperature	High, not uncommonly remittent	Slight temperature rise
Weakness	Marked in the majority of cases	Slight or absent
Coryza	May be absent; often occurs on the second or third day of the disease	Marked from the very onset of the disease
Cough	Is not a constant symptom, usually dry	Usually observed with excretion of mucopurulent sputum
Immunity	Elaborated	Not elaborated in diseases of bacterial origin; elaborated in diseases produced by adenoviruses
	Laboratory Data	
Blood	Leukopenia (beginning with the second or third day of the disease), relative lymphocytosis, monocytosis, decrease or absence of eosinophils, neutropenia, toxic granulation of neutrophils; in the majority of cases, sedimentation rate is normal.	No characteristic changes, sometimes slight leukocytosis; not uncommonly, the sedimentation rate is accelerated
Hemagglutination reaction with pharyngeal washings	Frequently positive	Negative
Increase of antibodies to influenza virus	Observed in the majority of cases	Not observed
Rhinocytologic examination	Presence of columnar epithelial cells in the impressions from the inferior nasal con-	In the absence of chronic nasal disease columnar epithelial cells are rarely observed in impressions

Table 1 [continued]

cha during the first few days	from the inferior nasal concha

Until recently it was believed that acute catarrhs of the respiratory passages are associated with conditionally pathogenic bacteria which inhabit the mucous membranes of the upper respiratory passages; under the influence of catarrhal and other factors which weaken the resistance of the body (particularly, the local resistance of the mucous membranes of the upper respiratory passages), these bacteria (staphylococci, streptococci, pneumococci, influenza bacilli and others) multiply actively, producing local inflammatory processes and general signs (fever, intoxication).

This view of the acute catarrhs of the respiratory passages was shaken after the discovery of the adenoviruses (Rowe, Huebner, Gilmore et al, 1953) and following the establishment of their part in the etiology of epidemic outbreaks of acute diseases of the respiratory tracts (Hilleman, Werner, 1954). Afterwards, it was possible to detect a whole group of adenoviruses (the other name for them is adenopharyngo-conjunctival viruses or, in abbreviated form, APC viruses) and other agents of viral origin which produce influenza-like diseases. The majority of them has been discovered by means of the use of the method of tissue cultures, in which they produce characteristic cell injury -- a cytopathogenic effect (Sabin, 1956). Analysis of this group of viruses, as of the entire heterogeneous group of the acute catarrhs of the respiratory passages, goes beyond the realm of this book and should be the subject of a special monograph.

3. Study of the Pathogenesis, Clinical Aspects and Epidemiology of Influenza

Following the discovery of the causal organism of influenza many problems were studied of pathogenesis and immunity in this disease. It was established through the investigations of Smorodintsev, Drobyshevskaya and Ostrovskaya (1938) that the influenza virus produces, along with local afflictions (Francis and Stuart-Harris, 1938), a marked depression of the defensive forces of the body, in connection with which secondary infection produced by the conditionally pathogenic microflora of the respiratory passages, plays an important part in the pathogenesis of both

influenza and the complications which follow it. Afterwards, Zakstel'skaya (1953) studied the causes of intoxication in influenza and the mechanism of the injurious affect of the influenza virus on the human body in greater detail (development of leukopenia, suppression of the phagocytic activity of white blood cells, etc.). The principles of the pathogenetic therapy have been worked out on the basis of current knowledge of the pathogenesis of influenza.

A considerable number of investigations of the past two decades has been devoted to the study of immunity in influenza. Through these investigations (Andrewes, Laidlaw, and Smith, 1935; Burnet, Lush and Jackson, 1939; Smorodintsev and Shishkina, 1936-1946; Solov'yev and Parnes, 1946; Zil'ber, Baydakova and Blyumberg, 1946; and Zakstel'skaya, 1953) it was established that of chief importance in the development of a specific immunity to influenza are the accumulation of antibodies in the blood and nasal secretion, the development of resistance of susceptible tissues of the respiratory tract as well as certain physiologic mechanisms: the temperature reaction, destruction of the virus in the blood, excretion of it from the body by the kidneys.

In connection with the development of the physiologic teaching of I. P. Pavlov many problems of pathogenesis and immunity in influenza have been critically revised in recent years on the basis of it.

In contrast to problems of etiology, pathogenesis and immunity in influenza, the study of which has been put on a firm basis only since the discovery of the influenza pathogens, the clinical aspects and epidemiology of influenza have been studied in detail even before the establishment of the virus etiology of the disease, whereby much has been done in this area by Russian scientists (see the summary of Kantorovich, 1954).

Many problems in the clinical aspects of influenza have been studied in the past century. It is particularly essential to note the investigations of Russian scientists made at the end of the past century in connection with the influenza pandemic of 1889-1890, which have maintained their importance to the present time (Filatov, 1903). Such important investigations were made during the influenza pandemic of 1918-1920 (Glinchikov, 1922). At the time, considerable new information on the clinical aspect of influenza was obtained in subsequent years until the most recent time. The results of these investigations have been generalized in the monographs of Rossiyskiy (1942, 1949) and Epshteyn (1949), as well as in numerous works published in the periodical press.

Many problems in the epidemiology of influenza have also been studied in the past century (Vasil'yev 1889). The historical-epidemiological data were generalized at that time by Hirsch in the monograph mentioned above.

The influenza pandemic of 1918-1920 produced an abundance of new material for epidemiologic observations. Specifically, during these and subsequent years the modern concepts of the sources of infection, mechanism of transition of the infective nidus, and the significance of immunity in the epidemiology of influenza were worked out conclusively. At the same time, a wealth of data was accumulated concerning the morbidity and mortality rate statistics of influenza, the characteristics of distribution of influenza epidemics among the population, the morbidity rate of various occupational population groups, the significance of the seasonal factor in the epidemiology of influenza, etc. The most important data on the epidemiology of influenza have been generalized in the works of Sadov (1927), Dobreytser (1935), Khotsyanov (1944), Gromashevskiy and Vayndrakh (1947), Sergeyev (1950), Romashenko (1953) as well as in the works of foreign investigators, Dopter and Lavergne (1925). At the same time, certain problems in the epidemiology of influenza have recently required a radical revision (Zhdanov, 1954).

On the basis of a study of the etiology, pathogenesis, immunity, clinical aspects and epidemiology cardinal problems of diagnosis, specific therapy and prophylaxis of influenza have been solved in recent years.

Of the methods of laboratory diagnosis serologic diagnosis, based on the neutralization of the hemagglutinating properties of the influenza virus by the sera of those who have had the disease and a cytologic analysis of smear-impressions from the nose (Kolyaditskaya, 1948) have obtained greatest application. Less accurate results have been obtained through the use of serologic reactions for the detection of the influenzal antigen in the nasopharynx -- the hemagglutination and the complement-fixation reactions (Smorodintsev and others, 1953). Methods of isolating the influenza virus and culturing it outside the human body have definitely advanced recently. In sum, the laboratory diagnosis of influenza at the present time is not any more poorly developed than that for a number of well-studied bacterial infections.

4. Study of the Therapy and Prophylaxis of Influenza

Along with methods of symptomatic therapy used previously, methods of treating influenza with chemotherapeutic preparations and antibiotics have been worked out in

the past 10-15 years the aim of which is the suppression of conditionally pathogenic microflora and the prevention of complications. Of the specific methods of treatment serum therapy, which was given a sound basis by Smorodintsev in 1938, has been used. The chemotherapy of influenza proper, like thechemotherapy of other virus infections, remains the least developed division of the problem to date.

Attempts were made to obtain effective live influenzal vaccines for the purpose of immunizing people soon after the discovery of the influenza virus (Smorodintsev, 1937; Chalkina, 1938; Burnet, 1937; Chenoweth, 1936 and others). Abroad, and later also in the USSR, efforts of research workers were directed toward obtaining vaccines from killed virus (Smorodintsev, Ansheles and Orlova, 1943; Solov'yev and Sokolov, 1948; Francis, 1943) for subcutaneous use. The subcutaneous or intracutaneous route of application of influenzal vaccines, further than which foreign investigators have not as yet gone, has been considered without promise in the USSR, and the main efforts of investigators have been directed at a search for methods of increasing the efficacy of live vaccines for immunization through the upper respiratory passages.

The first models of live vaccines obtained in the USSR (Smorodintsev, 1938-1948) were produced from the lungs of white mice. Afterwards, a method of obtaining highly immunogenic allantoic strains was worked out in detail (Zhdanov and Solov'yev, 1952; Smorodintsev and Chalkina, 1948-1953; Fadeyeva, 1953), as was also the technology of preparation of the dry vaccines (Sokolov, 1953; Smorodintsev and Chalkina, 1953). The preparations of live vaccines obtained proved to be quite effective on epidemiologic testing of them and were accepted as the principal measure for the prophylaxis of influenza. It should be noted that large-scale prophylactic immunization of the population against influenza with live vaccines was first used in the Soviet Union, and priority in this respect goes entirely to Soviet virology.

Therefore, at the present time many divisions of the big and complex problem of influenza have been satisfactorily solved. As a result of many years of investigation, which have been carried on particularly extensively and actively in the past two decades, the pathogens of influenza have been discovered and a study made of their biological properties; many problems of the pathogenesis and immunity have been clarified, and the clinical aspects and epidemiology of influenza have been studied. Practical medicine has at its disposal methods of diagnosis, therapy and specific prophylaxis of influenza. The prophylaxis and treatment of influenza are being formulated on a solid

scientific basis at the present time.

The main problem now is the most rapid possible incorporation of the methods and measures of diagnosis, therapy and prophylaxis of influenza into practice and a perfection of them along with the further scientific development of the influenza problem.

A reduction in the influenza morbidity rate and a prevention of the development of epidemics of it constitute an immediate and very actual problem of Soviet public health.

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SECOND CHAPTER

ETIOLOGY

5. Classification and Nomenclature of Influenza Viruses

The classification of influenza pathogens among other viruses has been discussed repeatedly, particularly in connection with the publication of systems and determinations of viruses.

Horsfall et al., (1940) suggested including in the nomenclature of influenza viruses only the pathogens of diseases which are transmitted from man to man (not from animals), designating each new serologic type of virus by the Latin letters "A," "B," "C," etc.

Holmes (1948) separates the pneumotropic viruses into a separate genus to which he also refers the influenza pathogen. His system cannot be considered correct, because the causal organism of parotitis, which is similar to influenza viruses in its biologic properties, has not been included in this genus, and at the same time the virus of fox encephalomyelitis, which is similar to the pathogen of rabies, has been included in the group. It should be noted that Holmes considers the viruses of influenza "A" and "B" independent species, and has named them, respectively, Tarpeia alpha and Tarpeia beta.

Zhdanov and Korenblit (1950) put the influenza viruses into the genus Pneumophilus. Ryzhkov (1950) puts them into the genus Pneumotropus, including approximately the same viruses in this genus. In Zhdanov's table of viruses (1953) the genus Pneumophilus includes the pathogens of influenza, swine influenza, mumps, Asiatic fowl pseudoplague, and certain other viruses. Along with the genus Enterophilus the influenza viruses are included in the family Pneumophilaceae, which in its turn and together with the families Gamaleiaceae and Botkinaceae form the order Gamaleiales.

In summary, the classification of the pathogens of influenza has the following form:

order Gamaleiales type vira genus Pneumophilus
family Pneumophilaceae class Zooviralia

The serologic types of influenza viruses described

to date by the majority of research workers are considered to be varieties. However, not all investigators agree with this. Thus, Zhdanov and Solov'yev (1951) believe that the causal organisms of influenza "A" and "B" are independent species, even though similar. Gorbunova (1952, 1953), who has worked out a classification of influenza viruses, somes to the same conclusion. In conjunction with her and based on her data, Zhdanov (1953, 1954) has suggested that the following species of influenza viruses be distinguished:

1. Pneumophilus gripposus A, the causal organism of type "A" human influenza.
2. Pneumophilus gripposus B, the causal organism of type "B" human influenza.
3. Pneumophilus gripposus C, the causal organism of type "C" human influenza.
4. Pneumophilus suis, the causal agent of swine influenza.

There are antigenic varieties within the limits of these species of which more will be said below. However, it should be pointed out that this classification is not accepted by all investigators. The majority considers the influenza viruses to be a single species represented by several serological types and a multitude of intratype variants.

In the light of the latest data certain corrections and additions need to be introduced into this classification.

The list of influenza viruses should be supplemented with a new species:

5. Pneumophilus gripposus "D," the causal organism of type "D" human influenza.

Virus A-prime should be considered a variety of the A virus, which will be discussed in detail below.

In accordance with these corrections the number of influenza species remains equal to five: A, B, C, D, and swine.

6. Dimensions and Filterability

It was established during the pandemic period of 1918-1920 that the influenza virus passes through Chamberland L_2 and L_3 and Berkefeld N filters (Odurua, 1936) and afterwards, through Berkefeld and Seitz filters (Shubladze and Gaydomovich, 1949).

Measurement of the size of the elementary bodies by different methods give approximately the same results. Friedewald and Pickels (1944) measured the dimensions of virus particles of influenza A and B by the methods of cen-

trifugation and filtration through colloidal membranes. In experiments with centrifugation of allantoic fluid containing the virus the dimensions of the virus particles were found to be about 80 millimu for the PR8 strain; about 85 millimu for the Lee strain. In experiments with filtration through colloidal Elford membranes the dimensions of the particles for these strains proved to be of the order of 80-135 millimu. Lauffer and Stanley (1944) determined the size of the influenza virus particles (PR8 strain) by methods of electron microscopy and centrifugation; the first method made it possible to determine thefact that the size of the particles amounts to 115 millimu (standard deviation, 15 percent); with the use of the second method the diameter of the particles was found to be 100 millimu. Previously, Elford, Andrewes and Tang (1936) determined the size of virus particles of the influenza A group (strain WS) and of swine influenza (Shope strain) by the method of ultrafiltration to be from 80-120 millimu and by the method of centrifugation Elford and Andrewes determined the size of the influenza virus particles to be from 87-99 millimu. Sharp and others (1944) used the method of electron microscopy and centrifugation for the purpose of determining the size of the virus particles; the dimensions of the particles were found to be 97.3 millimu with the use of the former method, and 99.8 millimu with the use of the latter method.

In Table 2 data are presented of measurements of the size of the elementary particles of influenza viruses obtained by various authors.

Here it is fitting to present the comparative data of results of measuring the size of influenza viruses by various methods, which have been generalized by Tovarnitskiy (1951) according to the data of various authors (Table 3).

As seen from Table 3, investigations of recent years which have been made by various authors give approximately the same results with the use of various methods. The range of size of the elementary bodies of the influenza viruses within limits of 80-120 millimu may depend not only on errors in the methods but also on the range of the virus particles themselves in different preparations.

Table 2

Size of Elementary Bodies of Influenza Viruses According to the Data of Various Authors

No	Author	Year	Virus Strain	Method of Measurement	Diameter of Virus Particles in Millimu
1	2	3	4	5	6
1	Elford et al.	1936	A(WS) Mouse, A (Hog)	Filtration	80 120
2	Elford a. Andrewes	1936	A(WS) Mouse	Centrifugation	87–99
3	Taylor et al.	1943	A(PR8) Allantoic	Electron Microscopy	77,6
4	Taylor et al.	1943	A(W), Hog Allantoic	Centrifugation E-lectron Microscopy	81,5 78,3
5	Sharp et a	1943	B(Lee) Allantoic	Centrifugation	100
6	Friedewald a. Pickels	1943	A(PR8), B(Lee)	Centrifugation	80–120
7	Lauffer a. Stanley	1944	A(PR8) Allantoic	Centrifugation	100
				Electron Microscopy	110–115
8	Stanley	1944	A(PR8, F12) Allantoic	Centrifugation	70
9	Lauffer a. Miller	1944	A(PR8, F12) Allantoic	Centrifugation	100
10	Sharp et al.	1944	B(Lee) Allantoic	Centrifugation	99,8
				Electron Microscopy	97,8
11	Friedewald a. Pickels	1944	A(PR8) Allantoic	Centrifugation	80
				Filtration	80–135
			B(Lee) Allantoic	Centrifugation	85
				Filtration	80–135
12	Dawson a. Elford	1949	A(PR8) Allantoic	Electr on Microscopy	90 ± 11,5
			B(Lee) Allantoic	Electron Microscopy	103 ± 8

Table 3

Size of Influenza Virus in Millimicrons

Name of Method	Type A	Type B	Author
Ultrafiltration	80 - 120		Elford
Ultracentrifugation	87 - 99		Elford
Adsorption on erythrocytes + ultracentrifugation	77,6	97,3	Sharp and Taylor
Same	80,1	100,4	Sharp and Taylor
Same	70	—	Stanley
Same	116	124	Sharp and Taylor
Same (from mouse lungs)	100	—	Knight
Electronmicrograph measurements	80—101	97—113	Sharp and Taylor
Same	115	—	Lauffer and Stanley
Same	—	123	Knight
Light adsorption	97	—	Oster

7. Morphology

The morphology of influenza viruses has been studied in detail at the present time. The elementary bodies of the influenza virus are represented in the electron microscope in the form of spheroidal or bodies of almost spheroidal shape with a diameter of about 100 millimu.

Fig. 1 gives us an idea of the morphology of influenza viruses. In the electron microscope the elementary bodies of influenza virus usually have a spheroidal shape or a shape similar to spheroidal, which is particularly clearly shown in photography with the use of shadowing (Fig. 2). The dimensions of the elementary bodies vary within comparatively narrow limits (Fig. 3).

The morphologic pictures in the preparations of influenza viruses may vary considerably; however, in the virus preparations which are adsorbed onto erythrocytes and in the tissue cultures accumulations of elementary bodies are frequently found in the form of chains or filaments (Figs. 4, 5). Sometimes, formations of bizaare shapes with irregular outlines are encountered.

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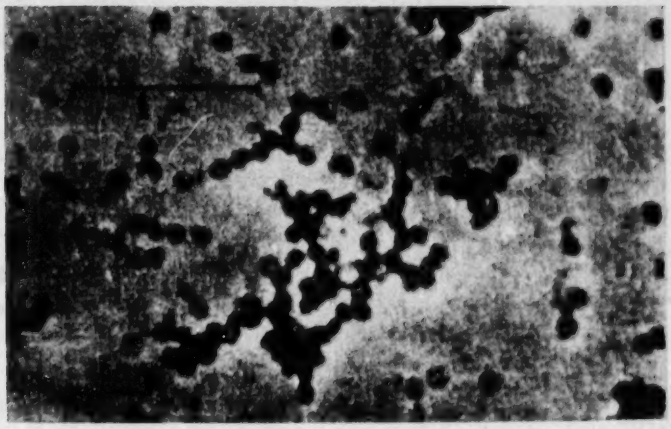


Fig. 1. Elementary Bodies of Influenza Virus Under Electron Microscope. The Lee Strain from Allantoic Fluid Following Double Adsorption, Elution and Centrifugation. Magnification 30, 100 x (Sharp and others, 1944).

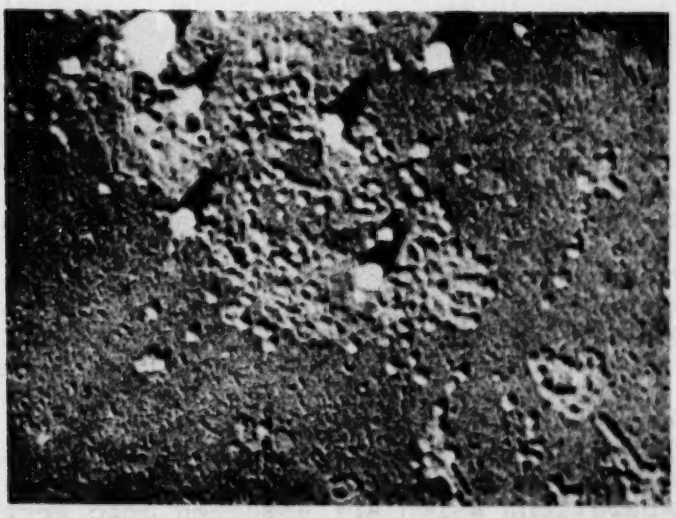


Fig. 2. Elementary Bodies of Influenza A Virus (PR8). Untreated Preparation From Allantoic Fluid. Shadowing With Chromium. Magnification 25, 200 x (Draganov, 1953). No of Particles in Each Hundred Particle Diameter in Millimu

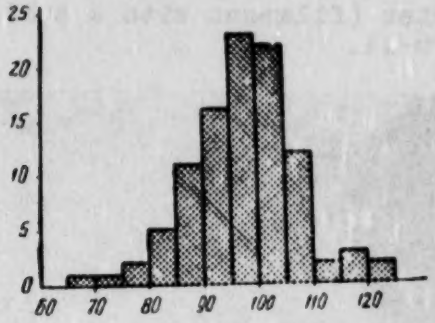


Fig. 3. Distribution of Sizes of Influenza Virus Particles on the Basis of Measurement of 100 Particles (Sharp and others, 1944).

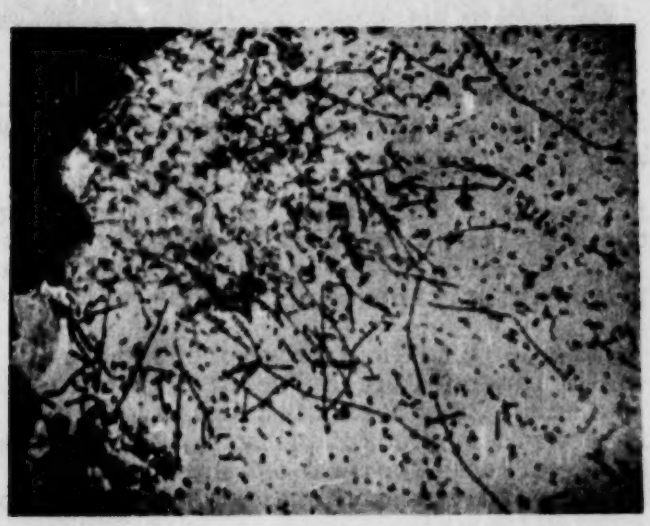


Fig. 4. Virus A-prime (FM1) in Tissue Culture According to the Maximow Method (Murphy and Bang, 1952).

Draganov (1953) and Draganov and Slepushkin (1954), using an original method of obtaining untreated virus preparations, found filament-like forms resembling a tennis racket or a disc with a process projecting from it in the allantoic fluid of chick embryos infected with influenza virus. Such structures are not found in the normal embryonic fluids. Some of the forms found by the author, which

he called NV bodies (filament with a swelling), are presented in Figs. 6-14.

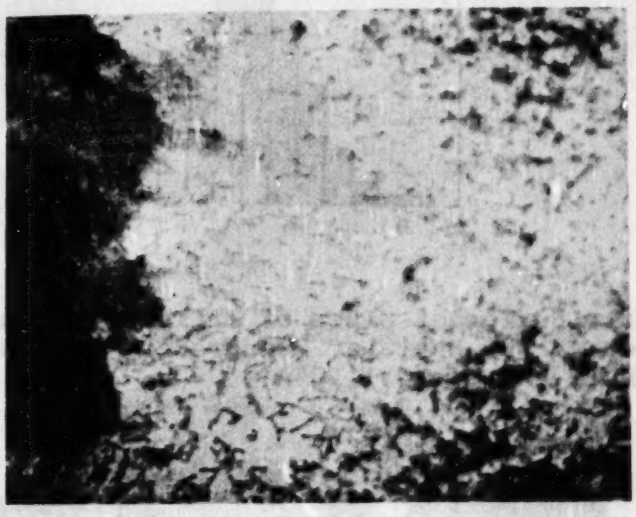
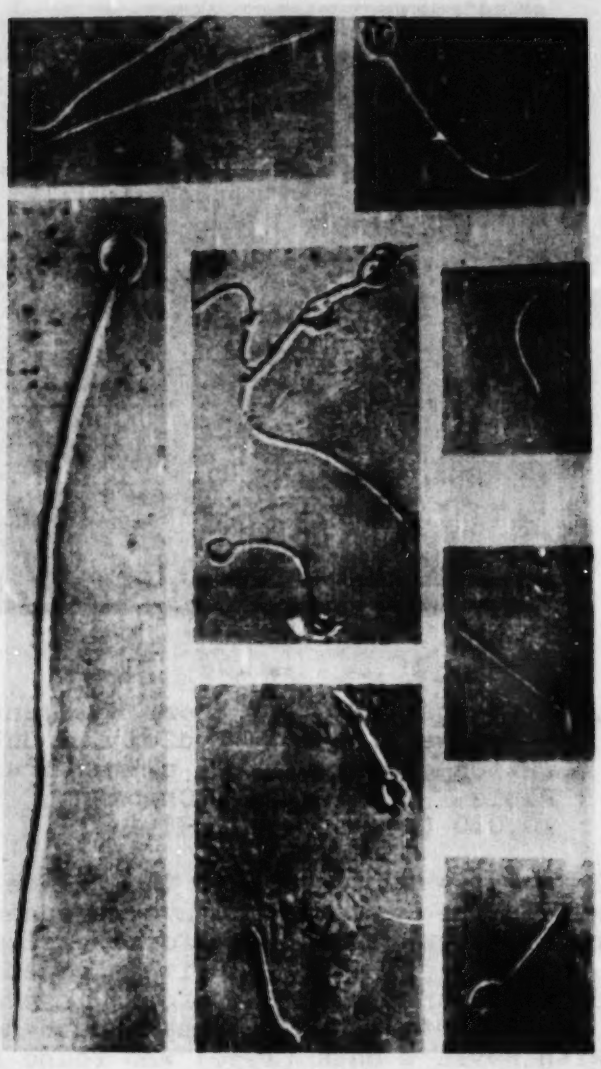


Fig. 5. Virus of Swine Influenza (V15) in Tissue Culture According to the Maximow Method (Murphy and Bang, 1952).

It is possible that some of the morphologic pictures are the results of different methods of treating the virus. No essential differences were found in the morphology of different strains of influenza virus (Magnus, 1954).

The doubt which has been expressed as to the viral nature of the filament-like structures observed in influenza virus cultures has been discredited at the present time. It is considered completely proved that the filaments are forms of development of the influenza virus in the tissues. The following data constitute proofs of this statement.

1. The filaments are absent in allantoic fluid of normal chick embryos and are observed with great constancy in embryos infected with influenza viruses.
2. Filamentous structures existing in tissues are different from the influenza virus filaments.
3. The filaments are not artefacts, because they can be seen in untreated preparations by means of a phase-contrast apparatus.
4. The filaments react with erythrocytes as elementary bodies, and in heated form they are not removed from the erythrocytes.



Figs. 6-13. NV Bodies (Filaments with Swellings) in Untreated Preparations of Influenza A-prime Virus Adsorbed onto Erythrocytes (Draganov, 1953)

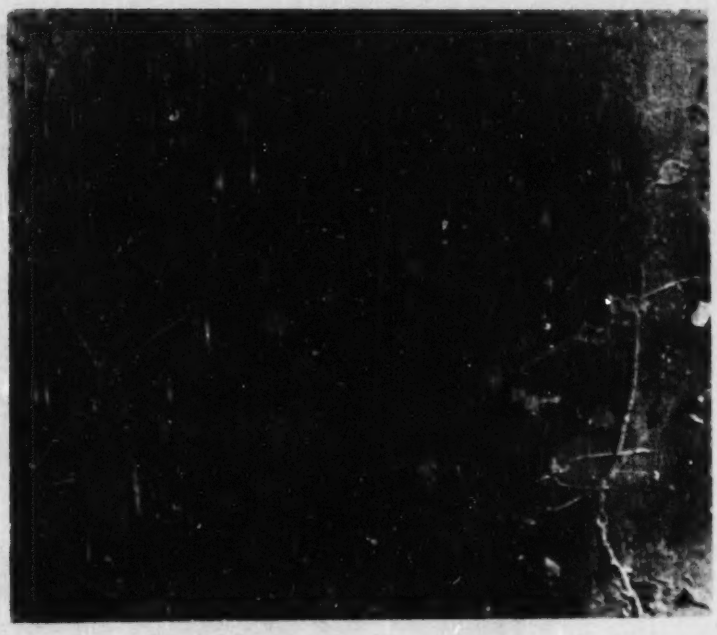


Fig. 14. Elementary Bodies, Short Filaments and Filaments With Swellings (NV Bodies) in Untreated Preparation of Influenza A-prime Virus from Allantoic Fluid. Dusting With Chromium. Magnification 10,000 (Draganov, 1953).

It must be supposed that the filaments appear during the growth of the virus under favorable conditions and that afterwards they can break down forming elementary bodies.

From this point of view it is expedient to compare the findings of these distinct forms in preparations of influenza virus mentioned, which have been described by Draganov, with Hoyle's data (1950) who found them in untreated preparations by means of a phase-contrast apparatus. In Figs. 15 and 16 Hoyle's drawings are presented; some of them are strikingly similar to the NV structures described by Draganov.

The method of silver staining with subsequent phase-contrast and ordinary microscopy has been used for the investigation of the influenza virus by Morozov (1953) and his co-workers, Merkulova and Kazanskiy (1950). According to the authors' data, it is possible by means of this method

to detect elementary bodies of influenza virus both in experimental infection of white mice and in smears from the contents of the nasal cavity of people sick with influenza. In Figs. 17 and 18, which have been reproduced from the work of Merkulova, Kazanskiy and Khamazyuk (1954), the morphologic pictures obtained with the use of this method are presented.

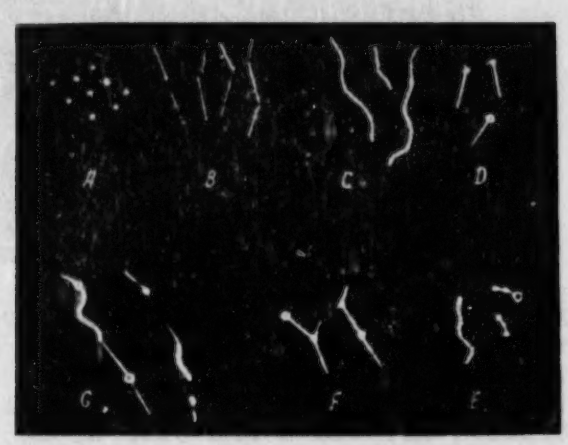


Fig. 15. Morphology of Influenza Virus in Native Preparations of Allantoic Fluid in a Dark Field Under a Phase-Contrast Microscope (Hoyle, 1950).

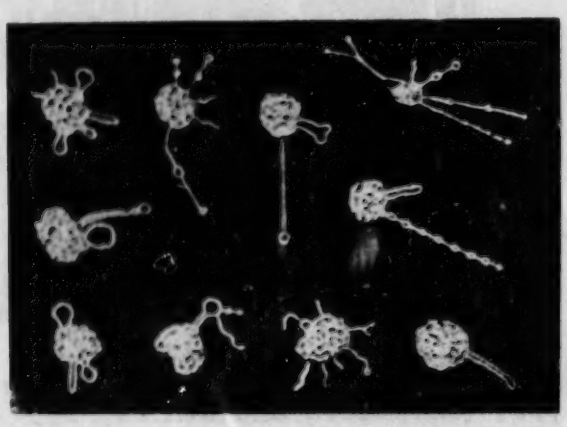


Fig. 16. Desquamated Allantoic Cells With Filamentous Structures (Influenza Virus?) in Dark Field Under Phase-Contrast Microscope (Hoyle, 1950).

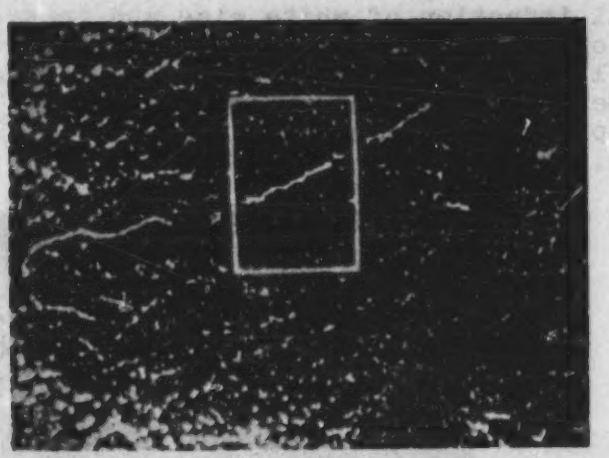


Fig. 17. Elementary Bodies of Influenza Virus With Silver Staining According to the Morozov Method. Negative Phase-Contrast Microscope of GOI [State Optical Institute]. Magnification 1,100 (Photo by Morozov, Merkulova and others, 1954).

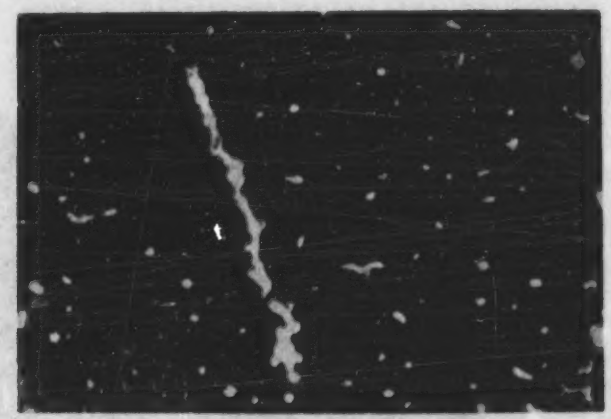


Fig. 18. Outlined Section of Fig. 17. Magnification 5,000 (Merkulova and others, 1954).

We shall return to the morphology of the influenza virus in the description of the hemagglutination phenomenon and the development of influenza virus in tissues.

8. Physico-Chemical Properties

As has already been mentioned, the influenza virus passes through the bacterial berkefeld VN, Chamberland L_2 and L_3 and Seitz filters. In the filtration of it through graduated Elford colloidal filters pores of the diameter of 80-120 millimu constitute the lowest limit through which the viruses will pass. Filters with pores of smaller diameter do not permit the influenza virus to go through.

The sedimentation constant has been determined repeatedly, chiefly with the aim of subsequent recalculation for the purpose of determining the dimensions of the elementary virus bodies. Lauffer and Stanley (1944) found a sedimentation constant for the PR8 virus A, expressed in Svedberg units ($S_{20} \cdot 10^{-13}$), equal to 718-725S; Stanley (1944) found a sedimentation constant of about 600S for PR8 (A) and F12 (A) strains; Friedewald and Pickels (1944) found a sedimentation constant of about 700S for the PR8 (A) strain and of about 800S for the Lee (B) strain; Taylor and others (1943) found a sedimentation constant of 662S for the virus of swine influenza; Sharp and others (1943), of 832S for the Lee (B) strain. Therefore, according to the data of investigators occupied in the determination of the sedimentation constant, the magnitude of the latter for influenza viruses should be taken as 700-800S.

In Makower's work (1953), magnitudes of the sedimentation constant for virus A of 700-742S, for virus B of 800-840S, and for the virus of swine influenza of 727S are presented on the basis of summarized data from the literature.

The problem of the magnitude of the sedimentation constant of influenza viruses has become complicated after the publication of the investigations of Chambers and Henle (1941). These authors found through experiments with centrifugation and diffusion of the influenza virus that about one percent of the virus in the lungs of infected mice and a somewhat larger number in the allantoic fluid have a sedimentation constant of about 30S, which corresponds to a particle diameter of about six millimu. Chambers and others (1943) showed that in an allantoic culture of the F12 (A) strain about 10 percent of the particles of spherical shape had a sedimentation constant of from 20 to 31S, which corresponded to a particle size of about 11 millimu and a molecular weight of about 650,000. These data were not confirmed by Taylor and others (1943), Sharp and others (1943) with regard to the PR8 (A) and Lee (B) strains. However, it was shown through the checking investigations of Stanley (1944) that there are two compon-

ents in an allantoic culture of an F12 (A) strain: one with a sedimentation constant of about 600S, associated with infectious activity of the virus, and the other with a sedimentation constant of about 30S which is not associated with infectious activity. In analyzing the factual material presented in these works of Taylor and others mentioned, stanley showed that even in the experiments of these authors about five percent of the virus consisted of slowly sedimenting particles of small diameter, and, therefore, the results of the experiments were inaccurately appraised by the authors themselves.

By means of fractional centrifugation Stanley separated both components and obtained each of them in a purified form.

Gard and others (1952) in checking investigations of allantoic cultures of virus strains PR8 (A) and Lee (B) in general confirmed these data, although they obtained somewhat different values for the sedimentation constants of these viral components.

We shall return to these data in the discussion of the investigations on the so-called "soluble antigen" of the influenza virus.

The density of the influenza virus has been determined repeatedly also. Elford and Andrewes (1943) obtained a value of 1.2 for the A virus; Lauffer and Stanley (1944) obtained a value of 1.18 for the PR8 (A) strain. Similar values have also been obtained for viruses A-prime and B. In the work mentioned above Makower presents average data of the densities of influenza viruses: A = 1.1 - 1.104; B = 1.1104; of swine influenza, 1.1.

Lauffer and Stanley obtained values of the specific volume for PR8 (A) strain within limits of from 0.78 to 0.85; Taylor and others (1943) obtained specific volume values of 0.84 for the swine influenza strain; Sharp and others (1943), 0.85 for the Lee (B) strain; and in other work (1944) a value of 0.865 was obtained.

An electrophoretic study has been made of influenza viruses by a whole series of authors.

Tovarnitskiy and Shishkina (1943), studying the electrophoresis of the influenza A virus in a Lepine apparatus, established the fact that the virus has a negative charge in its entire stability zone (pH of from 5.2 to 7.8); however, purification of it by this method is complicated, because the isoelectric point of the virus (pH equal 4.8-5.3) is below the acid zone of stability. The influenza virus may be reactivated from mixtures with specific immune serum by the method of electrophoresis.

Miller, Lauffer and Stanley (1944) showed that in

unpurified preparations of influenza virus (PR8) (A) strain obtained after centrifugation at 24,000 revolutions per minute there are two electrophoretically different components. One of them (comprising 10-20 percent of the material investigated) is a component part of the embryo; the other (which comprises 80-90 percent of the material) is the active virus with an isoelectric point of pH = 5.3. The same value was obtained both after the use of the method of microelectrophoresis according to the Northrop-Kunitz method and through determination in a Tiselius electrophoresis apparatus (Fig. 19). Similar data have been obtained also by other authors.

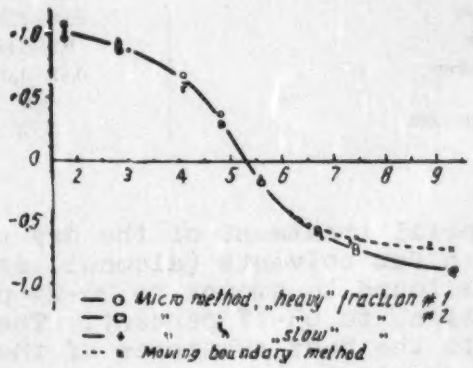


Fig. 19. Mobility and Isoelectric Point of Purified Preparation of Influenza A Virus (PR8). On the abscissa axis the values of pH are indicated: on the ordinate axis, the mobility in cm/sec/volt/cm · 10^4 (Miller and others, 1944).

The internal viscosity as determined in the Ostwald viscosimeter, according to the data of Lauffer and Stanley (1944), ranges within limits of 11.3-16.5.

9. Chemical Composition

The chemical composition of the influenza virus has been determined repeatedly by many authors (Sharp and others, 1943; Taylor and others, 1943; Knight, 1947; Friedewald and Pickels, 1944; Lauffer and Stanley, 1944, and others).

The most highly purified virus preparations contain about 60 percent water and about 40 percent dry matter.

The concentration of elements (in percentages) obtained through an elementary analysis of the dry matter is

presented in Table 4.

Table 4

Elementary Composition of Influenza Virus

Name of the Element	Concentration in %
Carbon	51,4—55,98
Oxygen	20—24
Hydrogen	8,08—8,16
Nitrogen	9,0—11,01
Phosphorus	0,87—1,07
Sulfur	0,97
Inorganic ash	3,13

After special treatment of the dry purified virus preparations with fat solvents (alcohol, ether), the lipoid fraction was found to amount to 20-24 percent; the non-lipoid fraction, to 65-77 percent. The lipoids are strongly bound to the body substance of the virus and cannot be obtained by simple extraction.

In Table 5 the composition of the lipoid fraction is presented (in percentages of the dry weight).

Table 5

Composition of Lipoid Fraction of Influenza Virus

Name of Lipoid	Concentration in %
Neutral fats	5.7 7.64
Cholesterol	5,1 5,67
Phospholipids	10.2 10,64

In the fatty fraction the phosphorus amounts to

about 52 percent of the total phosphorus found in the substance of the virus. The relationship of nitrogen to phosphorus in the phospholipid fraction is equal to 2:1, which is characteristic of the diaminomonophosphatides.

The A virus is richer in cholesterol; the B virus is richer in neutral fats (Tovarnitskiy, 1951). After the extraction of the lipoids the biological activity of the virus disappears, and the agglutinative activity is partially reduced.

Carbohydrates comprise 9.3-10 percent of the dry weight of the virus. They are represented by polysaccharides composed of mannose, galactose and glucosamine molecules.

The polysaccharides which are not included in the virus nucleic acid are bound to protein in a ratio of 1:3. The concentration of glucosamine in the A virus is equal to 4.1-4.5 percent; in the B virus, to 2.9-3.5 percent. The problem of the origin of this component is not clear, since it is also possible in the normal allantoic fluid to find polysaccharide with a content of glucosamine amounting to 4.8-5 percent (Tovarnitskiy, 1951).

Up to 68 percent of the dry weight of the virus is constituted by proteins. Nucleoproteins contain ribonucleic and desoxyribonucleic acid which constitute three to four percent of the weight of the virus.

According to newer data, influenza A viruses contain 2.1 percent yeast nucleic acid and 2.3 percent thymonucleic acid (Tovarnitskiy, 1953); the influenza B viruses contain about 3.7 percent thymonucleic acid (Tovarnitskiy, 1951).

The composition of nucleic acid preparations isolated from purified influenza virus preparations (strain PR8) was the following: nitrogen 9-10.8 percent, phosphorus 4.9-6.4 percent, carbohydrates (mannose, galactose, glucosamine) 33-37.5 percent, ribonucleic acid 44.8-47.6 percent, desoxyribonucleic acid 19-50 percent.

In the analysis of 2,465 milligrams of the purified virus preparation 20.95 milligrams of phosphorus, 240 milligrams of nitrogen, 17.5 milligrams of ribose and 150 milligrams of carbohydrates of the glucose type were found (Knight, 1947).

No essential differences were found in the chemical composition of different influenza virus strains (PR8, Lee, swine), and the same applies to the study of strains adapted to various hosts (mice, chick embryos).

In Table 6, which is taken from Tovarnitskiy's review (1951), data are presented on the chemical composition of viruses A and B compared with the normal allantoic fluid.

Table 6

Chemical Composition of Influenza Virus Isolated from Allantoic Fluid (in percentages of the dry weight)

Substance (Component) Determined	Type A	Type B	Normal Allantoic Fluid Component
Carbon	53,2	52,7	55,3
Nitrogen	10,0	9,7	9,5
Carbohydrates	12,5	13,1	7,0
Lipoids	23,4	22,4	35,0
Phospholipids	11,5	11,2	23,4
Cholesterol	7,0	3,7	6,5
Fats	5,1	7,2	6,9
Non-lipids	77,5	76,4	66,5
Proteins	65,0	63,6	40,7
Carbohydrates	7,3	9,4	9,5
Thymonucleic acid	(1,5)[1]	(1,2)	(?)
Yeast Nucleic acid	(?)[2]	(?)	6--10

[1]Rough data
[2]Presence of the substance established but quantity of it not determined.

[1]Rough data
[2]Presence of the substance established but quantity of it not determined.

More distinct differences were found through an analysis of the amino acid composition of influenza A and B viruses (Knight, 1947). The corresponding data are presented in Table 7.

It is interesting to compare the results of the investigation of virus activity with these data. Calculations show that in purified preparations the quantity of virus corresponding to a single ID_{50} for chick embryos is contained in $10^{-11.2}$-$10^{-12.74}$ grams of the preparation, which in its turn corresponds to 6600 virus particles. The quantity of virus wh ch corresponds to two agglutinating red blood cell units is contained in $10^{-6.29}$-$10^{-6.5}$ grams of the preparation (Sharp and others, 1943). Infected allantoic fluid contains about 0.01 percent of the virus or about $2 \cdot 10^{10}$ virus particles in one cubic centimeter. The minimum infective doses for various strains of virus may differ considerably: for example, for the PR8 strain about 10 virus particles cause an infection of a

chick embryo and of mice; for the Lee strain 10 particles cause infection in the embryo and only 10,000 particles are needed to cause infection of mice.

Table 7

Amino Acid Composition of Influenza Virus in Percentages

Amino Acid	Type A	Type B	Normal Component of Non-Infected Allantoic Fluid
Arginine	5,0	4,0	3,9
Glutamic acid	7,7	6,2	6,1
Lysine	3,6	4,7	2,5
Tryptophane	1,1	0,7	0,7
Tyrosine	3,1	2,1	2,2
Glycine	2,5	2,9	1,8
Histidine	1,4	1,5	0,8
Alanine	2,5	2,6	—
Aspartic acid	7,4	7,3	6,2
Isoleucine	4,1	4,2	3,2
Leucine	5,3	5,5	4,3
Methionine	2,3	2,1	1,1
Phenylalanine	3,7	3,4	3,6
Proline	2,6	2,7	2,8
Serine	2,2	2,2	2,1
Threonine	3,7	4,0	3,8
Valine	3,4	3,2	3,2

Note. The influenza A virus contains 52 percent water; the B virus, 34 percent water (Tovarnitskiy, 1953).

For the purpose of characterizing the chemical composition of influenza viruses it should be stated further that, apart from the virus protein, the host proteins are included in the influenza virus particles. These proteins cannot be differentiated by chemical methods, and they are found by means of serologic reactions. Treatment of the virus isolated from mouse lungs with serum immune to normal mouse lung tissue leads to a partial precipitation of the virus. In a corresponding manner, after treatment of the virus isolated by allantoic fluid serum obtained by

means of immunization of animals with normal fluid also produces a partial precipitation of the virus. Purification of the virus preparations does not eliminate this phenomenon. The precipitation does not occur if the allantoic virus is treated with normal mouse lung antiserum or if the mouse virus is treated with allantoic fluid antiserum (Knight, 1947). In exactly the same way it is sufficient to passage the mouse virus once through allantoic fluid in order that it stop being precipitated by allantoic antiserum.

In evaluating these facts, Tovarnitskiy (1951) comes to the conclusion that the proteins of the cells in which the viruses are cultured are firmly bound to the virus particles. The virus particles, therefore, have their own protein, which is independent of the medium in which the viruses are cultured, and the protein of the medium in which they are cultured. The infective properties are associated with the former, the individual virus protein; therefore, the corresponding immune serum not only precipitates the virus but also neutralizes its infectious properties. In contrast to this, the normal tissue component antiserum does not neutralize the infective activity of the virus by precipitating it.

10. Purification and Concentration of Influenza Viruses

For the purpose of obtaining purified concentrated preparations of influenza viruses methods of ultracentrifugation, precipitation by chemical agents and adsorption onto erythrocytes are used (Tovarnitskiy, 1948).

Method of Ultracentrifugation. When high-speed refrigeration centrifuges are available this method is one of the ideal methods of obtaining purified virus preparations. The initial clarification of the allantoic fluid is accomplished in an ordinary centrifuge, after which the virus is precipitated in a vacuum, continuous-flow Sharples type refrigerated centrifuge, which can revolve at speeds of 50,000 revolutions per minute. In Stanley's experiments (1944) the crude allantoic fluid contained 287 agglutinating units of the virus per cubic centimeter; after centrifugation, up to 1,000 units per cubic centimeter; the virus yield reached 70 percent. The preparation contained up to 22,000 hemagglutining units of the virus per milligram of protein nitrogen; the infective dose for chick embryos (ID_{50}) was 10^{-14} grams. Less powerful centrifuges, which revolve at about 24,000 revolutions per minute, can also be used for the purification and concentration. Allantoic fluid is clarified in a cen-

trifuge at 4,000-5,000 revolutions a minute; then it is centrifuged at 24,000 revolutions per minute for 1.5-2 hours. The precipitate is dissolved in 0.1 molar phosphate buffer, clarified in the centrifuge at 4,000-5,000 revolutions per minute and again centrifuged at 24,000 revolutions per minute. The preparations contain about 3,800 hemagglutining units per milligram of protein, and their ID_{50} for chick embryos reaches 10^{-13} grams (Knight and Stanley, 1944).

These methods produce comparatively large quantities of the purified virus. Small quantities of the virus and the behavior of them are investigated by means of analytic ultracentrifuges (Friedewald and Pickels, 1944). Methods of separating the components with sedimentation constants of 600 and 30S have been described in Stanley's work (1944).

More detailed information concerning methods of obtaining purified preparations of influenza virus with centrifugation of the contained virus suspensions are available in the works of Taylor and co-authors (1943), Sharp and co-authors (1943, 1944) as well as Gard and co-authors (1952).

Gypsum, alums, ion-exchange resins and other substances are used as chemical adsorbents for the purification and concentration of influenza viruses.

Gypsum Method. This method was developed by Tovarnitskiy and Chalkina (1943). First a saturated aqueous solution of gypsum is prepared, the insoluble salts are allowed to settle, the transparent solution is poured off and used for the work (gypsum water). Using this gypsum water which has been cooled to 0°, a suspension is prepared containing the virus which is clarified by centrifugation at 3,000-4,000 revolutions per minute. A certain volume of the clarified fluid is transferred to a centrifuge tube, and 30 percent by volume of refrigerated alcohol is added to it in a slow stream with mixing, after which the fluid is placed in the refrigerator for an hour. The precipitate is separated by centrifugation; the supernatant fluid is poured off. For the purpose of elution of the virus the precipitate is dissolved in Ringer's solution of water at a pH = 7.4, mixing with a glass rod with a rubber tip. With this method of treatment the virus is purified of 70-80 percent of the inert proteins, and when treated twice by this method, 90-97 percent of the inert proteins are eliminated, and the virus is concentrated by 200-400 times.

Precipitation and Concentration With Alums (Bodily, Corey, Eaton, 1943). The virus-containing allantoic fluid

is mixed with 10-percent alum solution ($Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 12H_2O$) in different proportions for the purpose of determining the optimum precipitation conditions. The precipitate formed in the course of several seconds is centrifuged at 3,000 revolutions a minute for 20 minutes. The supernatant fluid is poured off, and the latter is washed by centrifuging three times in physiologic solution. The washed precipitate is diluted in physiologic solution 1:10 with respect to the original volume, and for the purpose of preservation merthiolate in a dilution of 1:10,000 is added. For the purpose of elution of the virus the precipitate is dissolved in 20 percent sodium citrate which is added to the required volume.

Kaolin Adsorption (Tovarnitskiy, 1948). Commercial kaolin is boiled for 20 minutes with an equal volume of dilute hydrochloric acid; the mixture is filtered through glass wool, washed with distilled water until the chloride ion is completely removed, transferred to a watch glass, dried at 100° and ground up into a fine homogeneous powder. A phosphate buffer prepared according to the Sörensen method (1/15 molar; pH = 6.2-6.4) is diluted with distilled water to 1/30-1/45 molar. A 1/600 molar amonia solution is prepared at pH = 7.6 as an eluate. The suspension of the virus which has been diluted in the buffer and freed of the coarse suspension by centrifugation is added to a 10-percent suspension in a kaolin buffer in a ratio of 1:5, carefully shaken up for 10-15 minutes, and left in the refrigerator for two hours; then, it is centrifuged for 20 minutes, the precipitate is washed with acidified (pH = 5.6-6.6) distilled water twice in the centrifuge. The wash water is poured off, the ammonia solution is poured onto the precipitate, shaken for 15 minutes and left in a refrigerator for 2-4 hours or for the night. After elution the fluid is centrifuged. The virus is contained in the supernatant fluid.

Of the other methods of chemical purification of the influenza virus note should be made of adsorption onto a cation-exchange resin (Miller and Rose, 1952), methanol precipitation (Pollard and others, 1949; Tamm, 1950), adsorption onto infusorial earth (Vylegzhanin, 1942).

Method of Freezing and Thawing. This method is based on the formation of a urate precipitate, from which the virus is eluted (Stanley, 1944). Allantoic fluid containing the virus is frozen for the night in cellophane bags with dry ice, after which it is thawed at room temperature for 2.5 hours; thereby, care is taken that the temperature of the fluid does not go above 3°. The melted fluid is centrifuged in a cold room at 3,000-4,000 re-

volutions per minute; the precipitate is taken up and dissolved in 0.1 molar phosphate buffer (pH = 7.0) for 30 minutes at a temperature of 23°. The undissolved precipitate is treated again by the same method after preliminary centrifugation of it.

Urate Method (Tovarnitskiy, 1948). The method is mentioned above -- precipitation of the virus on urates contained in allantoic fluid. Potassium urate (0.5 gram) is dissolved with normal potassium hydroxide solution (5-7 cubic centimeters); boiling water (100 cubic centimeters) is poured in; after cooling, it is filtered and carefully neutralized with hydrochloric acid to a pH = 7.8-8.0. The virus suspension is thoroughly mixed with the urate solution (1:10), centrifuged, and the clarified fluid is frozen for the night in a refrigerator. After thawing, the precipitate containing the virus is centrifuged, a phosphate (pH = 7.4-7.6) or acetate (pH = 5.5) buffer in a volume corresponding to the desired concentration (5-20 times) is added to it.

Concentration and Purification of Virus by Adsorption onto Erythrocytes. This method has been extensively used because of its simplicity and availability. The virus is adsorbed onto erythrocytes at a low temperature and eluted at 37°. The method described by Sokolov (1951, 1954) is presented below.

Twenty cubic centimeters of allantoic fluid cooled to 4° and containing the influenza virus is poured into each of several large centrifuge tubes; then, washed chick embryo erythrocytes are added so that their final concentration amounts to one percent. After a slight shaking of the mixture of the virus with the erythrocytes the test-tubes are placed in the refrigerator at 4-5° for two hours for the purpose of adsorption of the virus. Then, the suspension is centrifuged, and the supernatant fluid is removed. Two cubic centimeters of supernatant fluid, which constitutes 0.1 of the original volume of virus, is added to the erythrocyte precipitate. The erythrocyte precipitate is carefully shaken and the test-tubes are placed in a thermostat for two hours at 37° for the purpose of elution of the virus. At the end of two hours the red blood cells are separated by centrifugation for five to seven minutes at 1,500-2,000 revolutions per minute.

By determining the hemagglutinin concentration in the suspension before adsorption, (n) by titration, in the supernatant fluid after it (c) as well as in the eluate (k) the percentage of adsorbed virus can be determined, as can also the efficiency of concentration and elution of it. For this purpose it is necessary to determine the quantity of hemagglutinins (n-c) adsorbed by the erythrocytes and

then express the value found in percentages according to the formula:

$$(n-c)\cdot\frac{100}{n}\ .$$

The percentage of concentrated hemagglutinins is determined according to the following formula:

$$EK = \frac{k\cdot 100}{n\cdot 100},$$

where k is the concentration of hemagglutinins in the eluate.

Because the elution of the virus is accomplished in 1/10 of the original volume of the virus suspension used for the concentration and, therefore, some percentage of the virus contained in the original 10 cubic centimeters of virus is contained in one cubic centimeter of the eluate, the concentration of hemagglutinins in the original virus suspension (n) needs to be multiplied by 10 for the purpose of calculating the percentage of concentrated virus. By substitution of the values found into the formula, the percentage of concentrated virus is determined.

The percentage of eluted virus is determined according to the following formula:

$$E = \frac{k\cdot 100}{(n-c)\cdot 10},$$

where (n-c) times 10 is the quantity of virus adsorbed by the red blood cells from the 10 cubic centimeters of the original virus suspension.

For purposes of illustrating the effectiveness of this method of concentrating the influenza virus it is fitting to present data (Table 8) taken from the work of the author (Sokolov, 1954).

In the author's experiments the red blood cells adsorb 87-95 percent and elute 90-100 percent of the virus, which amounts to 80-90 percent of the original virus. The degree of purification of the virus reaches 50-70 percent.

An even better method of purification of the influenza virus is possible by means of a four-fold adsorption and elution of it onto erythrocytes (Sheffield, Smith, Belyavin, 1954).

Table 8

Effectiveness of Adsorption and Elution of Virus

Material	Hemagglutinin Titer			Infective Virus Titer		
	Type A	Type B		Type A	Type B	
	Strain					
	PR8	Weiss	Lee	PR8	Lee	Lee
Virus suspension before adsorption	1 : 1 280	1 : 320	1 : 320	$\cdot 10^{-7}$	10^{-4}	$\cdot 10^{-3}$
Virus suspension after adsorption	1 : 160	1 : 80	1 : 2	10^{-5}	10^{-2}	$\cdot 10^{-1}$
Eluate	1 : 10 240	1 : 2 560	1 : 320	10^{-8}	10^{-5}	10^{-4}

Yeremeyev's Method (Yeremeyev, 1952; Yeremeyev and Chalkina, 1953) is based on the utilization of natural stroma or of fixed erythrocytes for virus adsorption and elution in concentrated solutions of sodium chloride. Chick erythrocytes or guinea-pig erythrocytes are washed in a centrifuge with physiological solution, and four volumes of distilled water at a pH = 5.5 are added to the thick sediment; the suspension is shaken up and washed repeatedly with acidified water containing 0.2 percent sodium chloride and then with physiologic solution (pH = 7.2). The fixation of the erythrocytes is accomplished by means of addition of an equal volume of 50 percent formalin (or 75 percent alcohol) in five percent glucose to the sediment of freshly-washed erythrocytes. Before the experiment the erythrocytes are washed out in physiologic solution.

The adsorption of the virus is accomplished by means of addition of material to the stromata or fixed erythrocytes containing virus according to the calculation of 10 cubic centimeters of fluid for the quantity of stroma or fixed erythrocytes which corresponds to 0.2 cubic centimeter of the natural erythrocytes. After 15 minutes of the reaction the suspension is centrifuged, five cubic centimeters of five-percent sodium chloride solution is added to the sediment, and after 15 minutes the suspension is again centrifuged. The virus which has been eluted is in the supernatant fluid. A modification of this method has been proposed by Dreyzin (1953).

Combined Methods of purification and concentration of influenza viruses are used for obtaining highly purified preparations. Usually, a combination of differential

centrifugation is used in high-speed centrifuges with the adsorption and elution of the virus onto erythrocytes (Knight, 1936).

The degree of purification of the influenza virus with the use of various methods is indicated in Table 9, which is taken from the work of Tovarnitskiy (1951).

Table 9

Degree of Precipitation and Purification of Influenza Virus by Various Methods

Name of Method	Degree of Precipitation in %	Purification in %	Author
Single differential ultracentrifugation 24,000 revolutions per minute	97	93	Stanley
The same done twice with washing	70	97	
Adsorption (twice) onto erythrocytes	80	84	
Freezing and thawing of allantoic fluid	70	89	
Calcium phosphate adsorption	95	33	
Adsorption (once) onto erythrocytes	100	64	Sharp and Taylor
The same done twice	100	90	
The same with ultracentrifugation 27,000 revolutions per minute	70	96	
Gypsum	93 – 100	75 – 93	Tovarnitskiy
Urate	80 – 100	63 – 88	
Chemical	80 – 100	70 – 93	

As seen from Table 9, a high degree of precipitation of the influenza virus is achieved by various methods, but the best purification is given by differential centrifugation.

11. Resistance to the Effect of External Factors

The influenza pathogens belong to the group of viruses with comparatively little resistance.

In the experiments of Ostrovskaya, Chalkina and Olekhnovich (1938) the virus died after five minutes at 60° and after 30 minutes at 55°. The length of survival depended on the density of the mouse lung suspension: at 55° in a 0.1 percent virus suspension death occurred after three to five minutes; when it was mixed with a five-percent suspension of normal lungs, after 15-30 minutes.

Special investigations for the purpose of elucidating the resistance of influenza viruses to heating have been made by Gutman. Various strains of A-prime viruses were tested under equal heating conditions (YeM, C5 and LP); viruses of the B strain (Kri and Lee) were tested under the same conditions. In Fig. 20, data are presented on testing of viruses adapted exclusively to chick embryos; in Fig. 21, to white mice. In the latter case an investigation was made of the allantoic fluid of chick embryos after the first passage of a suspension of lung tissue. By this means the same experimental conditions were created. The heating was accomplished at 60°. The infectivity was determined by titration against chick embryos.

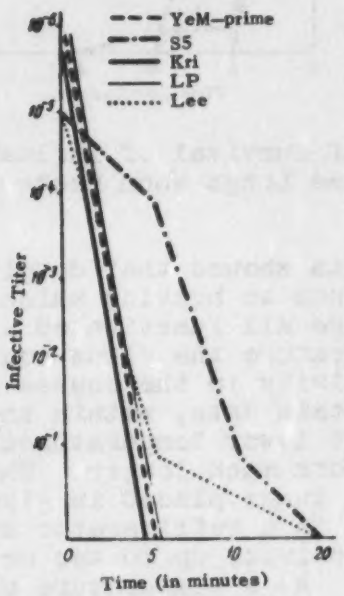


Fig. 20. Dynamics of Survival of Influenza Viruses Adapted to Chick Embryos When Heated at 60° (Gutman, 1954).

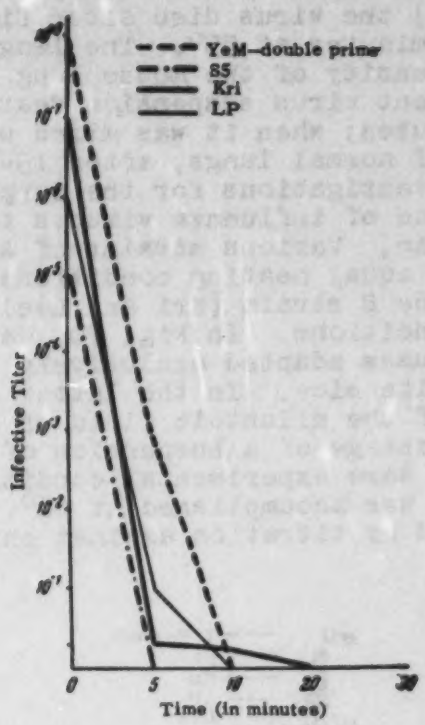


Fig. 21. Dynamics of Survival of Influenza Viruses Adapted to Mouse Lungs When Heated to 60° (Gutman, 1954).

The experiments showed that despite certain differences in the resistance to heating which exist in the various strains they were all inactivated after 20 minutes.

At room temperature the virus-containing suspensions lose their activity in the course of the first day, and according to certain data, within the first few hours (Sekretta, 1950). At lower temperatures the virus is preserved in a viable form much longer. The allantoic, amniotic fluid and mouse lungs placed in glycerin or suspended in a buffer solution in a refrigerator at a temperature of 4°, maintain their activity up to two or three months, gradually losing it. At a temperature of -20° or -76° the virus is preserved in a frozen state without marked loss of activity for several months, and complete destruction of it does not occur even after several years.

The virus activity may be preserved for a particularly long time when it is kept under conditions of low temperatures after lyophilic vacuum drying. The desiccated

virus in ampoules preserved at 4° shows a notable decrease in its infective titer only after 8-10 months, and that maintained at temperatures below zero shows a notable decrease in infective titer only over the course of several years. Therefore, at the present time lyophilic desiccation and subsequent preservation in the cold have come into extensive use both in work with museum strains of influenza virus and in the practice of preparation of influenza vaccines.

As an illustration of this situation we are presenting the data of Sokolov (1954) on virus titers in live dry vaccine when kept under various conditions (Table 10).

Table 10

The Influence of Environmental Temperature on the Influenza Virus Titer in a Dry Vaccine (According to the Infective Titer)

No. of Vaccine Series	Temperature at Which Kept	Time Elapsing After Desiccation						
		Days			Months			
		1	2	3	5-6	9	13-14	21
1	4°	10^{-7}	[1]	.	10^{-7}	.	10^{-6}	.
	18°	10^{-7}	.	.	10^{-7}	.	10^{-5}	.
2	4°	10^{-7}	.	.	10^{-7}	.	10^{-6}	.
	18°	10^{-7}	.	.	10^{-6}	.	10^{-6}	.
3	4°	10^{-7}	.	.	10^{-7}	.	10^{-7}	.
	18°	10^{-7}	.	.	10^{-6}	.	10^{-4}	.
4	4°	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}
	18°	10^{-6}	10^{-5}	10^{-5}	10^{-4}	10^{-5}	10^{-5}	10^{-4}
	37°	10^{-4}	10^{-2}	10^{-1}	.	.	.	.

[1] The period indicates that no examination was made.

It should be noted, however, that the infective titer for the chick embryo and the survival of the virus in the human nasopharynx decrease at different rates when kept in the refrigerator (4°). The data of Ritova and Zakstel'skaya (1954), obtained in our laboratory (Table 11), attest

to this.

Table 11

Change in the Properties of Live Influenza Vaccine on Keeping

Vaccine	Time Elapsing After Preparation	Titer of Active Virus in Chick Embryos	Survival Time (in hours) in the Nasopharynx of Various People											
			I			II			III			IV		
			48	72	96	48	72	96	48	72	96	48	72	96
Type A	12 days	10^{-9}	+	+	+	+	+	−	+	+	+	+	+	−
	1 month	10^{-9}	+	+	+	+	+	+	+	+	−	+	+	+
	3 months	$10^{-8.8}$	+	+	+	+	+	−	+	+	+	+	+	+
	6 months	$10^{-8.2}$	+	−	−	+	+	−	+	+	−	+	+	−
	7 »	$10^{-7.6}$	+	−	−	+	+	−	−	+	−	−	−	−
Type B	18 days	10^{-7}	+	−	+	−	−	−	−	+	−	+	+	−
	2 months	10^{-5}	+	−	−	+	−	−	−	−	−	−	−	−
	6 months	$10^{-4.5}$	−	+	−	−	−	−	+	−	−	−	−	−

From Table 11 it is seen that the capacity of survival and multiplication on the mucous membrane in the case of chick embryos falls off sooner than do the infective activity titers: the latter remain quite high even when the survival time of the viruses in the human nasopharynx falls off sharply.

The time that the influenza virus could be kept on surfaces and in the air was studied in detail in our laboratory by a worker of the Institute of Disinfection, Merekalova (1953). Thereby, it was shown that the influenza virus is more resistant than had been assumed previously (Ostrovskaya, Chalkina and Olekhnovich, 1938). When a large quantity of mouse-lung suspension infected with influenza virus strain M-12 (a) in a dilution of 1:200 (with respect to the original weight of the lung) was applied to a glass surface the virus maintained its infectious properties for mice up to 9-10 days in the light and up to 10-11 days in the dark; when the surface was infected with small quantities of the material the term of preservation of the

infectious activity of the virus was shortened to three days. At room temperature the virus maintained its activity up to three days on fabric surfaces; up to four days, on surfaces covered with an oil paint; up to one day, on plywood. Apparently, the death of the virus did not occur more quickly on rough surfaces, but it simply could not be extracted by washing, because it impregnated the fabric or wood. In air, it was possible to detect the virus four to 4.5 hours after spraying the suspension.

In the adsorbed form on animal or wood charcoal the influenza virus remained viable up to 14-30 days at room temperature and with access to air (Vylegzhanin, 1942).

Ultra violet irradiation rapidly destroys the influenza virus, particularly if it is distributed on the surfaces of objects. The part of the spectrum having a wave length of 253.7 Å possesses the strongest effect on the influenza virus; according to other data, from 313 to 500 Å (Smorodintsev, 1943, 1953). The efficacy of ultra violet irradiation depends on the concentration of the virus and the time of action of the irradiation. In the experiments mentioned, using the same source of irradiation, the virus on agar plates died after five seconds when its concentration was 50 MLD per cubic centimeter; after five minutes, when the concentration was increased by a 1,000 times.

The influenza virus is destroyed through the effect of ultrasound (Hamre, 1949).

When sprayed into the air the influenza virus is destroyed by oxidants of the chlorine and ozone type. When it reacts with low concentrations of active chlorine in the air (5-15 milligrams per cubic meter) inactivation of the influenza virus occurs in 30-60 minutes (Smorodintsev, 1953). Ozone acts equally destructively on the influenza virus: with a concentration of 0.25 milligrams per cubic meter the destruction of the virus occurs also after 30-60 minutes (Smorodintsev, 1953).

Vashkov and Astaf'yeva (1950, 1951, 1954) made a study of the viricidal effect of certain preparations on the influenza virus suspended in air. In their first work they showed that vapors of lactic acid in a concentration of five milligrams per cubic meter of air and glycerin vapors in a concentration of 10 milligrams per cubic meter of air possess considerable viricidal properties. In Fig. 22 data are presented concerning the death of mice in experiments with the spraying of influenza virus and the evaporation of glycerin. A similar picture was observed also after the evaporation of lactic acid.

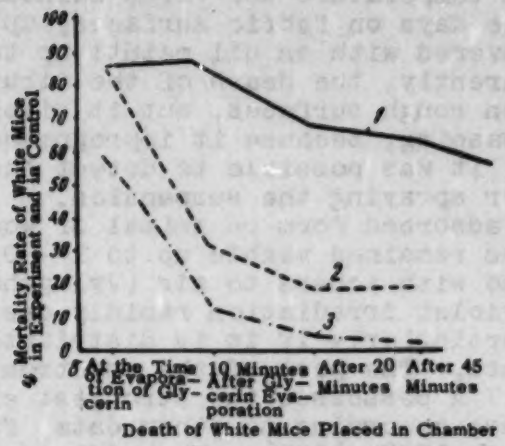


Fig. 22. Death of White Mice in Control and in Experiments With the Evaporation of 10 Milligrams of Glycerin per Cubic Meter After Spraying Influenza Virus (Vashkov, 1950).

1 -- death rate in control; 2 -- death rate of mice in experiments with spraying of virus 10 minuts after evaporation of glycerin; 3 -- death rate of mice in experiments with simultaneous evaporation of glycerin and spraying of the virus.

In the next work (1951) a study was made of another series of substances which were evaporated or sprayed into the air of a chamber into which the influenza virus was sprayed simultaneously or subsequently. The death rate or survival rate of mice which had been placed in the chamber served as the criterion of the effect of the substances on the influenza virus. Aside from lactic acid and glycerin, resorcin, hexylresorcin, pyrogallol (the latter preparation proved to be most effective) proved to be viricidal. DDT and pine oil aerosols were less viricidal.

In the last work (1954) the effect of certain organic acids was tested on the influenza virus in air. Of these, pyruvic, levulinic, glyceric, monochloracetic, dichloracetic, and trichloracetic acids proved to be effective in a concentration of 10 milligrams per cubic meter of air, preventing infection of mice in 77.4-92 percent of cases. Acetic, citric, isovaleric, N-butyric, formic and

capronic acids were not very effective.

The resistance of influenza viruses in liquid media and the influence of physical and chemical factors were also studied repeatedly.

In buffer solutions the infective activity of the virus is preserved longer than in distilled water and physiologic solution: the purified allantoic virus (strain PR8) at room temperature in distilled water or physiologic saline solution preserves its effective properties no more than four days, whereas in phosphate, borate or veronal buffer solutions it remains active up to 18 days (Knight, 1944).

The optimum reaction of the medium for preservation of the influenza virus activity is near neutral, although it may vary for different strains. According to the data of Ostrovskaya, Chalkina and Olekhnovich (1938), the optimum pH for the influenza virus is equal to 7.0-7.5. In an acid medium the virus is destroyed more quickly than in an alkaline medium.

In Miller's experiments (1944) the optimum pH for allantoic cultures of the PR8 (A) strain was 6.5-7.0; for the swine influenza strain, 7.0-7.9; for the Lee (B) strain, 7.9.

The isoelectric point for the influenza A virus is pH = 5.4 both for that isolated from infected mouse lungs and from the allantoic fluid. The isoelectric point of normal tissue components in the former case is pH = 4.2; in the latter case, pH = 2.3.

The influenza virus is not very resistant to disinfectants ordinarily used.

In ethyl alcohol the influenza virus dies after five minutes in a concentration of 70 percent and after 60 minutes in a concentration of 50 percent; the respective concentrations of mercuric chloride are equal to 1:500 and 1:2000; of phenol, five percent and three percent; of chlorine water, calculating for the active chlorine, more than 10 milligrams per liter; of formalin, 1:250 and 1:1000. The influenza virus is less resistant than the hemolytic streptococcus. At the same time, in lung emulsions the virus is relatively resistant to oxidants (potassium permanganate, hydrogen peroxide). In ether mixed in equal volumes with five-percent mouse-lung suspension infected with influenza the virus can be found after 24 hours; in the same mixture with chloroform, after three hours. Calcex [tablest containing urotropin salt and calcium chloride used in the treatment of initial stages of influenza] and urotropin in concentrations of up to five percent proved to be inactive with respect to the influenza virus (Ostrovskaya, Chalkina and Olekhnovich, 1938).

In three minutes the virus is inactivated by three percent phenol, 0.1 percent tincture of iodine, one percent Lugol's solution, potassium permanganate 1:1000, mercuric chloride 1:1000, 90 percent propylene glycol, and copper sulphate one percent (Dunham, McNeal, 1944).

The influenza virus is destroyed in allantoic fluid by oxidants heavy metal salts, mercurochrome, formaldehyde, and detergents (phemerol, roccal, dodecyl, sodium sulfate). Reducing agents act weakly on the influenza virus, with the exception of ascorbic acid, which destroys it even in a concentration of 0.05 N. Sodium sulfathiazol possesses a weakly inactivating effect on the influenza virus. Phenol destroys the virus in a concentration of 0.5 percent, but is not very active in lower, bactericidal concentrations. Glucose, ammonium sulfate, calcium chloride, sodium hyposulfite and arginine do not affect the influenza virus even in considerable concentrations (Knight and Stanley, 1944).

Chlorethylamines of the bis-(β-chlorethyl) amine type destroy the influenza virus, and in this process the toxic and infectious properties of the virus are the first to be inactivated. (Fong and others, 1953.)

Yeremeyev (1949) showed that urea and glucose in five- to 10-percent concentration do not produce any changes in the influenza virus; more concentrated solutions of urea (30-40 percent) disaggregate the influenza virus particles, inactivating them. The resistance of the virus is increased in 5-10 percent solutions of potassium chloride and is decreased in the presence of salts of the alkaline earth metals. Dialysis leads to a partial precipitation of the virus without any loss of its biological activity.

Preparations of the mustard gas type (bis-(β-chlorethyl) sulfide and other chlorethylamines) exert a definite effect on the influenza virus. They do not destroy the influenza virus immediately, but depending on the dosage used and the exposure time they first impair its infectious activity, and then its toxicity, interference properties, the power of agglutinating erythrocytes and of being eluted from them, and, finally, its antigenic structure. Incidentally, a similar phenomenon is observed with the careful use of ultra violet rays (Henle and Henle, 1947). This problem has been studied in detail by Fong and co-authors (1953).

The material on the effect of antibiotics and chemotherapeutic agents on the influenza virus will be analyzed in the chapter on the treatment of influenza.

12. Methods of Isolation and Culture of Influenza Viruses. Collection and Treatment of the Material

Since the time of discovery of the influenza pathogen methods of isolation and cultivation of it outside the human body have been used: passages in susceptible animals, passages in tissue cultures, and passages in the developing chick embryo.

The first strains of the influenza virus (type A) were isolated from African ferrets by means of infecting the animals through the nose with washings from the nasopharynx of patients: the WS strain (Smith, Andrewes and Laidlaw, 1933), the PR8 strain (Francis, 1934), the A36 strain (Smorodintsev and others, 1936) and others. However, this method has not been extensively applied for a number of reasons. The ferrets are bred with difficulty under laboratory conditions, and therefore it is difficult to obtain a large number of these animals. The infection progresses in them briefly and non-lethally, in connection with which it is not possible to obtain large numbers of the virus from the ferrets. Incidentally, the animals are exceedingly susceptible to the influenza virus and keeping them requires strict isolation to avoid infection from strains existing in the laboratory.

Beginning with 1936, after Zil'ber and coworkers (1937) and Francis and Magill (1937) established the existence of susceptibility of white mice to direct infection with excretions of people sick with influenza, these animals began to be used widely for the isolation and subsequent study of the influenza virus. White mice proved to be a convenient experimental model for the study of influenza; however, they are not well suited for isolation of the virus. Isolation of the influenza virus in mice is accomplished by means of a series of blind passages, during the course of which the virus is adapted to the bodies of the mice and become pathogenic for it, producing the development of a fatal pneumonia. Not all the strains adapt quickly and readily to the body of the mouse, as a result of which the percentage of positive results from infection of mice with the original material obtained from patients is small. During the course of serial intranasal passages an activation of latent pneumotropic viruses and bacteria not uncommonly occurs the carriage of which is widespread in mice, in connection with which a weakly-adapted influenza virus is frequently lost. Although the droplet route of transmission of the virus from sick mice to healthy ones does not occur, as a rule, because the deep-lying parts of the respiratory tract (lungs) are in-

volved, during the course of serial passages a contamination of the room may occur with material containing the influenza virus, and the mice may become infected with it. The possibility of intralaboratory infection of fresh mice has been shown repeatedly (Andrewes and others, 1944; Kravchenko and Sekretta, 1947, 1949; Parnes, 1950; Magill and Jotz, 1952). The method was worked out for isolating the virus from chick embryos.

The method of tissue cultures was suggested for isolating the virus of the common cold in 1932 and then also for the purpose of isolating the influenza virus by Dochez, Mills, Kneeland (1933, 1936) in the Maitland modification, as well as by Francis and Magill (1935) and a number of other authors (Smith, 1935; Lozovaya, 1942). The Li-Rivers modification for influenza virus culture was used by Packalen (1946). The method of roller-tubes for culture of the influenza virus was used by Pearson and Enders (1941). However, before 1941 no direct methods of detecting the virus in culture were known, in connection with which tissue cultures did not become widely used. The modification of the tissue culture method after Carrel for the purpose of isolating and studying influenza was used successfully by Fadeyeva (1952), and at the present time has begun to be used chiefly for obtaining vaccine strains of influenza viruses. By means of this method strains of the A-prime virus -- Eva (Fadeyeva, 1952) and Krav (Ritova, 1952) viruses have been successfully isolated.

Culture on a developing chick embryo has become the principal method for isolating and studying influenza viruses after Hirst (1941), McClelland and Hare (1941) showed the property of influenza viruses of producing erythrocyte agglutination, and this reaction began to be used as a method for detecting the influenza virus in material under investigation.

For the successful isolation of influenza viruses the treatment of virus-containing material is of great importance. It has particular importance in working with chick embryos, which are readily contaminated with foreign microflora which cause the death of the embryo and which do not allow the possibility of development of the influenza virus.

At first, the viruses were isolated by means of a preliminary filtration of the material. Afterwards, it was suggested that the influenza viruses be isolated from unfiltered nasopharyngeal washings to avoid loss of the virus when it is adsorbed onto filters (Rickard, Thigpen, Crowley, 1944). At the present time, antibiotics are utilized for the suppression of the bacterial flora (penicil-

lin, streptomycin).

For the purpose of isolating the virus, washings from the pharynx are collected in a sterile jar containing glass beads; cotton tampons are dropped into it after taking mucus from the nose. The washing is accomplished with 10 cubic centimeters of physiological solution. The jar containing the wash fluid is carefully shaken up so that the contents may be homogenized by means of the beads and so that the clumps of mucus may be broken up. After this, the fluid is clarified by means of aspirating it with a Pasteur pipette or with a syringe through a cotton tampon which is in the jar.

Penicillin is added to one cubic centimeter of the clarified washing, and after a 10-15 minute contact this material is introduced into the amnion of 10-11-day chick embryos.

In the isolation of the virus from tissues a 10-percent homogeneous suspension of the material is prepared from the tissues after grinding it up in a mortar and adding sterile fluid (physiological solution or buffered solution, bouillon, normal serum, etc.). Penicillin or a mixture of penicillin with streptomycin is added to one cubic centimeter of the fluid which has been clarified by means of centrifugation in a quantity determined by the degree of contamination of the material with bacterial flora (quantity of antibiotics from 100-1000 units per cubic centimeter of the material).

In sick children the nasopharyngeal mucus is removed with a cotton tampon which is preliminarily moistened in five cubic centimeters of sterile hormonal bouillon. The contents of the test-tube (washing or tampon containing nasopharyngeal mucus in hormonal bouillon) is brought to the laboratory in a frozen state. Thawing of it is accomplished before the examination. Sometimes, it is expedient to combine washings from three to five patients simultaneously in a single group. The total quantity of the combined washing (15-25 cubic centimeters) is concentrated with the use of one-percent sterile guinea pig erythrocytes. The concentration is accomplished on ice for two hours. After this the washing is centrifuged at 1500 revolutions per minute for 15 minutes; the supernatant fluid is poured off, and penicillin, streptomycin and one cubic centimeter of sterile physiological solution are added to the precipitate. In this way a concentration of the washing of 15-25 times is obtained. The concentrated washing with the antibiotic are kept on ice for 30 minutes, and then chick embryos are infected intraamniotically.

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13. Cultivation of Influenza Viruses on the Chick Embryo. Brief Data on the Anatomy and Physiology of the Chick Embryo

The hen's egg is enclosed in a lime shell, which has pores through which gas exchange is accomplished. Under it lies a membrane or the egg-shell membrane which is a necessary constituent of both fertilized and unfertilized eggs. To the present time it is not known whether any latent viruses or antibodies are present in the chick embryo. The embryonic tissue is a sensitive medium for the cultivation of viruses. For this purpose the embryonic membrane, which is rich in tissues of embryonic epithelium, is of special importance.

The chick embryo develops from three embryonic layers -- ectoderm, mesoderm and entoderm. Afterwards, tissues and organs of the embryo are formed from them.

On Fig. 23 (Ivanov, 1937, 1945) a four-day embryo is pictured developing at one of the poles of the yolk sac. Along with the development of the body of the embryo two extraembryonic membranes develop from the ectoderm: the amnion and chorion (or Serosal membrane).

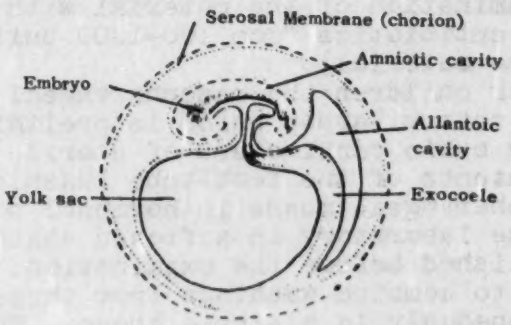

Fig. 23. Schematic Section of Four-Day Chick Embryo Key: ____ entoderm: ---- ectoderm: •••• mesoderm (Ivanov, 1937, 1945).

The amnion is constituted by a sac filled with perienbryonic fluid in which the body of the embryo rests. The amnion is in the immediate vicinity of the embryo, and the amniotic membrane is the inner primary fetal membrane proper. It is lined with ectodermal epithelium and is covered on the outside with a layer of mesoderm. The amniotic fluid, which is at first a simple physiological solution of

salts, is afterwards enriched with protein. The amniotic cavity is connected with the contents of the albuminoid sac, and after 12 days of development of the embryo the albumen begins to penetrate directly into the amniotic cavity. The quantity of amniotic fluid amounts to one cubic centimeter, on the average.

The chorion or serosal membrane is applied directly to the shell membrane, and is in contact with the outer air through the pores of the egg shell. The outer surface of the serosal membrane consists of monolayered ectodermal epithelial tissue which is supported by mesodermal tissue constituting the inner surface of the chorion. During the course of development of the embryo the chorion fuses with the outer wall of the allantoic sac, forming the chorioallantoic membrane.

In the early stage of embryonic development a projection grows out of its hind-gut -- the allantois. The allantois is a hollow organ-sac which arises from intestinal entoderm with a mesodermal outer layer. On growing out of the body of the embryo the allantois grows into the extraembryonic cavity (exocoele), passes between the amnion and chorion and surrounds the embryo together with the yolk sac, forming a secondary fetal membrane.

The allantois grows exceedingly rapidly. Its maximum growth occurs from the fourth through 13th day of development of the embryo, when it is converted from an intestinal diverticulum into a strong membrane which extends along the entire inner surface of the egg shell. The allantoic sac develops in two directions. On one side, it fuses with the mesodermal surface of the amniotic and yolk sacs with the mesodermal surface of its own inner wall, and on the other side it grows in the direction of the egg shell, fusing with the chorionic mesoderm with the mesodermal surface of its own outer wall. Part of the allantoic sac, fusing with the chorion, comes to be called the chorioallantoic membrane, which consists of three layers. The inner surface, which is directed into the cavity of the allantoic sac, consists of a single layer of cuboidal cells of entodermal epithelium. The middle layer is mesodermal tissue of the fused chorion and allantois, where a thick network of blood and lymphatic vessels develops. The outer surface of the chorioallantoic membrane is covered with a very thin layer of squamous ectodermal epithelial cells. In places, the capillaries of the mesodermal layer are applied directly to the shell membrane.

The chorioallantoic membrane carries out the function of the respiratory organ of the embryo. The oxygen exchange is accomplished in blood vessels of the membrane through the shell pores. However, in the last stages of

development of the embryo the chorioallantoic membrane atrophies; the air enters the pores of the shell, and the young chick begins to breathe with its lungs one or two days before it hatches out.

The allantoic sac is an excretory organ. At first, it is filled with a transparent fluid, which is a simple physiological salt solution; afterwards, the allantoic fluid is enriched with urates as well as substances which contain phosphorus and nitrogen. Beginning with the 12th-13th day of embryonic development the allantoic fluid becomes progressively more turbid, and after cooling the formation of a precipitate, consisting mainly of uric acid salts, may be observed in it.

The maximum quantity of allantoic fluid is accumulated on the 11th-13th day of embryonic development, reaching, on the average, 6-6.5 cubic centimeters. After this, its quantity decreases, and at the time the chick hatches none remains in the egg. During the period between the 7th and 12th day of development the reaction of the allantoic fluid is slightly alkaline; later, with a reduction in its quantity and the accumulation of urates, the reaction changes to acid at a pH = 6.0.

In Fig. 24 (Beveridge and Burnet, 1946) an embryo is shown with its cavities and membranes on the 10th-12th day of development. It is shown that the chorioallantoic membrane was formed from the chorion (solid line) and the allantois (broken line). At the point end of the egg the allantois, after attaining the edges of the chorion, continues on to the surface of the amniotic and holk sacs, forming the allantoic fluid which keeps the body of the embryo from direct contact with the chorioallantoic membrane and egg shell.

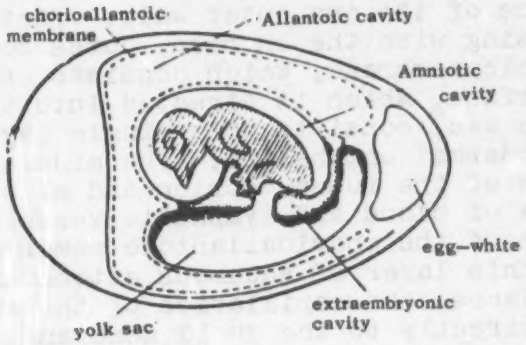

Fig. 24. Schematic Representation of Membranes and Cavities of 10-12-day Chick Embryos (Beveridge and Burnet, 1946).

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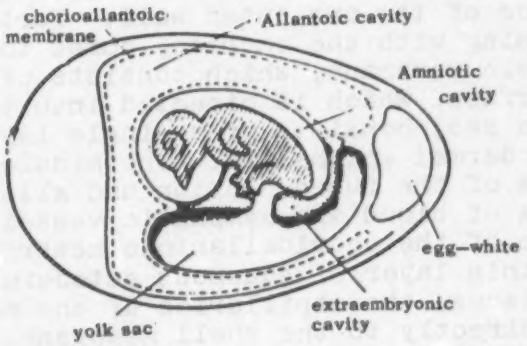

Fig. 24. Schematic Representation of Membranes and Cavities of 10-12-day Chick Embryos (Beveridge and Burnet, 1946).

The membrane in which the yolk sack lies plays an essential part in the cultivation of the viruses. The yolk sac is connected with the body of the embryo by a narrow yolk stalk inside which courses an entodermal canal which connects the yolk-sac entoderm with the intestinal embryonic entoderm.

During development the yolk sac is gradually adsorbed from the surface by the activity of the yolk-sac entoderm. The products of its assimilation enter the blood of the yolk-sac vessels, which carry them into the body of the embryo. As a result of absorption the yolk sphere decreases in size, the yolk sac covering it comes together, forming entodermal folds or villi which penetrate into the depth of the yolk sac and through which the absorption of the central parts of the yolk sac occurs.

Toward the end of embryonic life the yolk sac becomes fragile and atrophies, and the yolk is converted into a small sphere which is drawn into the body of the embryo, constituting a reserve supply of nutrient substances during the first few days of life of the chick.

In Fig. 24 the arrangement of the yolk sac is shown in a 10-12-day embryo. At this period of development the yolk sac occupies approximately one-half of the central portion of the egg; in the other half is the embryo.

The egg white in a 10-12-day embryo is an insignificant component part of the egg. It is displaced toward the pointed end of the egg by the embryo. The egg white is not an active medium for virus multiplication.

14. Cultivation of Viruses Using Various Methods of Infecting the Embryos

For the purpose of culturing the viruses fresh, recently laid fertilized eggs are used. Eggs in which the shell has become contaminated are discarded, because the possibility exists of penetration of the bacterial flora through the shell pores. Eggs which have been kept for a long time are also unsuitable, because they undergo drying, which involves an increase in the permeability of the shell.

In the practice of culturing influenza viruses embryos are used from the age of three or four days to the age of 13 days, depending on the method of infecting the embryo and depending on the greatest degree of susceptibility of various tissues of different ages to the given virus strain. Therefore, for the purpose of the infection embryos of different ages may be used in accordance with the conditions of the experiment planned. Usually, 10-12-day embryos are used for cultivation of influenza viruses.

After infection the embryos are incubated again for various periods of time -- from several hours to several days depending on the rapidity of multiplication of the given virus in the embryonic tissues. For example, an adapted influenza virus requires 48-72 hours for optimum multiplication, and an unadapted virus may achieve its optimum development at even later periods of time.

According to certain data in the literature a lower temperature is required for incubation of the embryos infected with the B virus, namely, 35-36°. Our practice of cultivating the influenza virus has shown that the B virus multiplies better at 35°, with a three-day incubation period after infection.

With any method of cultivation the egg shell over the site of injection of the virus material is first treated with alcohol and flamed; after that, it is treated with a weak alcoholic solution of iodine (until a pale yellow color is attained) and reflamed. After treatment of the egg shell, in some cases it is pierced with a lancet or a hole of the necessary size is drilled into it and in other cases a cut is made over the air sac with sterile scissors.

Cultivation on the chorioallantoic membrane is based on the capacity of the virus for multiplication in cells of ectodermal epithelium which cover the membrane from the outside. Infection of the membrane with certain viruses is accompanied by the formation of visible foci of pathology.

The technique of infecting the embryo on the surface of the chorioallantoic membrane consists of the following. By means of candling, the position of the embryo and the boundaries of the chorioallantoic membrane are determined; this can be determined easily by the branching of the blood vessels. For the purpose of infection of the chorioallantoic membrane 11-12-day embryos are used, that is, embryos in which the membrane is maximally developed.

We are presenting a description of one of the methods. At a site corresponding to the best development of the membrane a little window is filed out in the egg shell; the egg shell over the air sac is pierced together with the shell membrane. At the place where the little window has bee filed out the shell membrane is separated from the adjacent chorioallantois. Thereby it is necessary to be careful of injury to the blood vessels and the formation of hemorrhages. For this purpose a small slit is made in the shell membrane, onto which a drop of sterile physiological solution is applied. After this, by means of a small rubber nipple a little air is aspirated through the puncture made over the air sac. Then, by gravity, the drop

of fluid falls onto the surface of the chorioallantoic membrane, and air enters the space formed through separation of the shell membrane from the chorioallantois. A shift of the air sac occurs. An artificial air space is created over the surface of the chorioallantoic membrane in place of the natural one, which through the absorption of air is filled with the contents of the egg. Virus material is applied to the chorioallantoic membrane which appears (Fig. 25).

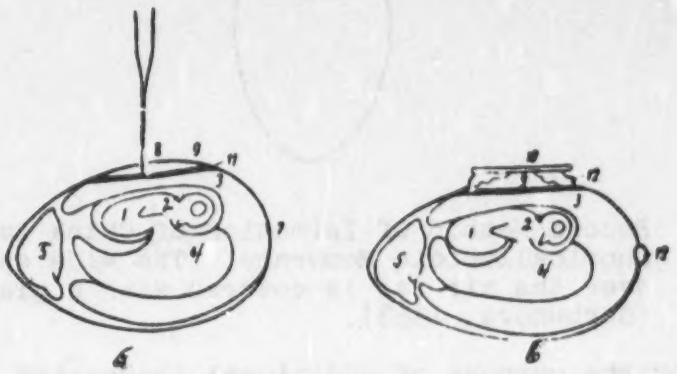

Fig. 25. a and b. First Method of Infection of Chick Embryo on Chorioallantoic Membrane (Zakstel'-skaya, 1954).

1 -- embryo; 2 -- amniotic cavity; 3 -- alantoic cavity; 4 -- yolk sac; 5 -- white; 6 -- air space; 7 -- puncture into egg shell for exit of air from air space; 8 -- little window in egg shell; 9 -- artificial air space; 10 -- cover glass; 11 -- cavity made by separation of shell and chorioallantoic membranes; 12 -- paraffin. Site of infection is shown in black.

After the infection the little window in the egg shell is covered with a cover glass or strip of cellophane and sealed hermetically with paraffin, as is also the opening over the air sac. The egg is transferred to an incubator in such a position that the little window is on top.

In another method of infecting the embryo on the surface of the chorioallantoic membrane no artificial air space or sawing-out of a little window is required. The shell is cut around over the air sac with scissors, the shell membrane is grasped with a fine forceps and carefully separated from the chorioallantoic membrane over an area of approximately one square centimeter. A drop of the virus material is applied to the chorioallantoic membrane

which appears. The opening in the shell is covered with a glass cover which is fastened onto the egg shell with paraffin (Fig. 26).

Fig. 26. Second Method of Infection of Chick Embryo on Chorioallantoic Membrane. The wide opening over the air sac is covered with a glass cover (Gorbunova, 1953).

For the purpose of additional incubation the eggs are put with the cover up into holders containing pockets.

For the purpose of extracting the chorioallantoic membrane which contains the virus culture the egg is incised at the optimum incubation times established for various viruses.

For the purpose of macroscopic examination of the membrane it should be washed twice in physiological solution and, after immersing it in a Petri dish containing physiological solution, it is examined against a black background. On cultivation of the adapted influenza virus isolated foci of pathology of different sizes or areas of thickening of the membrane which consist of fused foci may be seen. Depending on the conditions of distribution of the virus the foci may be located on the sections of the membrane which are far from the site of inoculation of the virus.

Pieces of chorioallantoic membrane are examined histologically. For the purpose of the histological study not only transverse sections through the thickness of the membrane but also total preparations which represent pieces of flattened membrane are of interest. As a source of virus the infected chorioallantoic membrane is used in the form of a suspension in physiological solution. For this purpose themembrane is cut with scissors into pieces of 0.5-

one square centimeter and is ground up in a sterile mortar with sand or broken up to a consistency of a homogeneous gruel in a jar with glass beads to which physiological solution or another solvent has been added, using a weight or volume calculation.

Infection of the Allantoic Cavity is a particularly valuable contribution to the method of cultivating viruses on the chick embryo. After multiplying in cells of the entodermal epithelium, the virus enters the allantoic fluid, accumulating in it in high concentrations.

The quantity of fluid in the infected allantoic sac increases notably; thus, after infection with the influenza virus it is possible to collect 7-10 cubic centimeters of fluid from a single egg, which is essentially a pure culture of the virus. The infected allantoic fluid of a 12-13-day embryo contains less than 0.1 percent protein and is suitable for basic experimental work even without any further treatment.

The method of embryonic infection of the allantoic cavity is exceedingly simple. The boundaries of the air sac and the location of the body of the embryo are determined by means of a preliminary candling and noted with pencil. In the center of the blunt end of the egg the egg shell is punctured with a drill or with a lancet. By means of a syringe and a needle material for culture is injected through the opening to a depth of two to three millimeters below the limit of the air sac. A Pasteur pipette may be used instead of the syringe (Fig. 27). Five-tenths of a cubic centimeter of fluid may be injected into the allantoic cavity; usually, 0.1-0.2 cubic centimeter is injected. The opening into the egg shell is poured over with fused paraffin. The eggs are placed into egg holders with the blunt end up and are transferred to the incubator.

The infection of the allantoic cavity can sometimes be performed conveniently after removal of the shell over the air sac. In such a case the material is injected beneath the visible chorioallantoic membrane. The opening is covered with a glass cover, as shown on Fig. 26.

This method is used in order to take samples of allantoic fluid repeatedly during the period of incubation with the aim of studying the dynamics of multiplication of the virus or a change of its chemical composition.

The obtaining of allantoic fluid from eggs infected by puncture is accomplished after cutting the egg shell over the air sac with scissors.

The fluid is aspirated with a Pasteur pipette, which is passed through the chorioallantoic membrane into the cavity. In puncturing the membrane areas free of blood ves-

sels should be selected so as not to produce bleeding.

Fig. 27. Method of Allantoic Infection (Zakstel'skaya, 1954). The key is the same as for Fig. 25.

Allantoic fluid collected with an admixture of blood is less active as a result of the adsorption of the virus onto the embryonic erythrocytes. In order to avoid bleeding the eggs which are to be incised are chilled for one to two hours in the refrigerator.

On cultivating the virus in the allantoic cavity the presence of virus in the allantoic fluid may be determined as early as during the course of the dissection of the egg by utilizing the hemagglutination reaction. For this purpose a drop of allantoic fluid is mixed with a drop of blood (10-percent suspension of chick or guinea-pig erythrocytes) on a glass slide or in a Petri dish. According to the rate and degree of the reaction it is possible tentatively to determine the quantitative concentration of the virus in the fluid. The hemagglutination reaction during the dissection of the egg may be accomplished also by means of mixing allantoic fluid with the blood which comes from damaged chorioallantoic blood vessels of the same embryo.

In the process of yolk-sac infection use is made of young three-six-day embryos. In this period, the embryo with its membranes constitutes a small appendage of the yolk sac. At the same time, the yolk-sac membrane is an already developed organ with actively developing absorptive functions of its entodermal epithelium.

The technique of yolk-sac infection is very simple. A puncture of the shell is made in the center of the blunt end of the egg over the air sac. The virus material is inoculated into the center of the egg, penetrating to its very center with the syringe needle or Pasteur pipette, that is, approximately a distance of 2.5 centimeters from the outer surface of the shell (Fig. 28).

Fig. 28. Method of Yolk-Sac Infection (Zakstel'skaya, 1954).

The incubation of infected eggs is continued until the embryo dies (from 3 to 10 or more days depending on the activity of the virus and the sensitivity of the embryo to it).

During the course of the cultivation samples may be taken from the yolk sac with the aim of determining the accumulation of virus in its contents. This is done through a puncture made in the shell by aspirating the yolk with a pipette or a syringe with a needle. However, it should be noted that multiplication of the virus cannot always be proved by its presence in the yolk. In the isolation of new strains the influenza virus may be found either in the yolk sac membrane alone or in any organ of the embryo, for example, in the trachea.

The dissection of the eggs infected in the yolk is accomplished by the same method as for an infection of the allantoic cavity by puncture. For the purpose of investigating the yolk-sac membrane it is necessary to separate the yolk sac as a whole and wash it free of yolk. The in-

ner entodermal surface of the yolk sac covered with villi is of particular significance.

For the purpose of biological determination of the existence of the virus a suspension should be prepared in physiological solution from ground-up yolk membrane tissue and utilized as a source of the virus for specific reactions and passages.

In view of the fact that with yolk-sac infection the embryo itself is readily infected the virus should also be sought in the organs, cavities and membranes of the embryo.

In the injection of virus material into the amniotic cavity a direct infection of the organs and tissues of the embryo or of the internal entodermal surface of the amniotic sac is intended. Thus, after the injection of throat washings of influenzal patients it may be assumed for purposes of primary isolation of the virus that a direct infection of the trachea of the 12-13-day embryo occurs, which at this time is making swallowing movements as a result of which amniotic fluid is entering not only the esophagus along with the virus but also the still non-functioning respiratory organs (trachea, lungs).

The infection into the amniotic cavity involves a generalization of the infection, since the virus involves the embryo itself. After passing through the body of the embryo the virus is excreted into the allantoic fluid where a massive multiplication occurs. Thereby, cases are possible where accumulation of virus in the amniotic fluid is negligible in comparison with its quantity in the allantoic cavity.

The amniotic cavity infection of embryos is possible at the age of seven days and at the age of 13-14 days. Thereby, the experimenter takes into consideration the duration of the incubation period, the degree of the tissue differentiation, their selective susceptibility and the existence of certain functions in the already developed embryonic organs at the age of 12-14-days.

The amniotic-cavity infection of the embryos may be accomplished principally by two methods: 1) by egg-shell puncture, and 2) extraction of the amnion to the outside.

Infection by shell puncture is accomplished in a darkened cubicle supplied with a simple ovoscope for carrying on the entire work with illumination.

A metal box without a bottom and with a roof 12 x 12 centimeters and a height of 10 centimeters may be used as an ovoscope which can be conveniently sterilized. In the wall of the box there is an opening for an electric light bulb socket; the light bulb is located inside the box. A round opening with a four centimeter diameter is made in the roof.

The egg is inserted into the opening with its blunt end up, the location of the embryo is determined by its shadow against the shell. Previously prepared virus material is introduced through a puncture in the shell made in the center of the blunt end using a Pasteur pipette which has been brought to a fine point. The introduction of pipette is accomplished with a vigorous jerk in the direction of the body of the embryo so that the movement of its shadow from contact with the point is noticeable (Fig. 29). In order to make sure that the point has penetrated into the amniotic cavity or has entered the body of the embryo and not simply moved it a movement is made in which the embryo follows the direction of the pipette toward the center of the egg. Then, an attempt is made to inject material by means of blowing air into the Pasteur pipette. If the blowing is somewhat difficult but it is nevertheless possible to inject about 0.1 cubic centimeter of material this indicates that the end of the pipette is in the amniotic cavity. The amniotic cavity is usually stretched from the presence of fluid in it and is immersed in allantoic fluid and is not able to distend much. If on insufflation of the material no obstacle is felt and fluid from the pipette is introduced freely it must be consi dered that the end is in the allantoic cavity, which dis tends freely because of the air space. Then, note should be made that material was injected into the allantois.

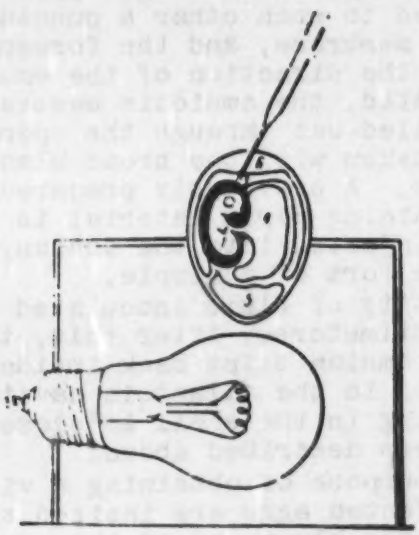

Fig. 29. Amniotic Sac Infection, First Method (Zakstel'-skaya, 1954).

If on insufflation of the material fluid practically does not leave the pipette it may be considered that the point has entered the body of the embryo. Then a slight backward movement of the pipette is made, and the area into which the inoculation has been made is judged by the pressure needed for injection of the material.

In addition to this pressure sensation in the Pasteur pipette the embryonic shadow, which is fixed in the event the pipette enters the amnion and is not connected with movements of the Pasteur pipette in the event it is located in the allantoic cavity, serves as a control. The opening in the egg shell is covered with fused paraffin.

Another method of infecting the embryo in its amniotic cavity is based on the exteriorization of a portion of the amniotic sac, as shown in Fig. 30. Thereby, the shell over the air sac is incised, as it was for chorioallantoic-membrane infection. The manipulation of exteriorization of the amnion is performed with two pipettes. A forceps with fine limbs is taken in the right hand; a depilsatory forceps with smooth flat expansions at the ends is taken in the left hand. A small piece of the shell membrane (about 0.3 square centimeters) is peeled off the chorioallantois in a place which may be assumed to be closest to the embryo between the branching vessels using the fine forceps. The embryo is clearly seen through the exposed section of chorioallantoic membrane. Then, with the same fine forceps in the right hand and with its limbs tightly appressed to each other a puncture is made in the chorioallantoic membrane, and the forceps is passed under the membrane in the direction of the embryo. Then, the limbs are separated, the amniotic membrane is grasped with the forceps, pulled out through the opening in the chorioallantois, and taken with the broad-blade forceps which is in the left hand. A previously prepared Pasteur pipette or syringe containing virus material is taken in the empty right hand and injected into the amnion, which has been drawn out in the form of a nipple.

The quantity of fluid inoculated can vary from 0.05 to 0.2 cubic centimeters. After this, the forceps is released, and the amnion slips back inside the egg occupying a normal position in the allantoic cavity.

The opening in the shell is closed with a glass cover, as has been described above.

For the purpose of obtaining a virus culture the amniotically infected eggs are incised through an opening in the shell at the blunt end of the egg. After infection which has been produced by puncture of the shell over the air space is cut around with scissors, and in the case of

infection under the glass cover the latter is removed.

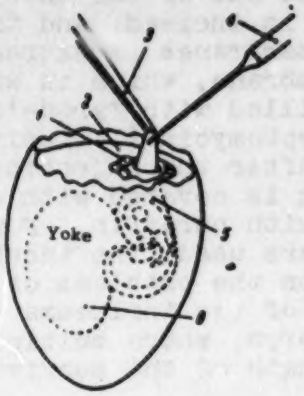

Fig. 30. Infection of Amniotic Sac, Second Method (Gorbunova, 1953).

1 -- exposed section of chorioallantoic membrane; 2 -- shell membrane; 3 -- forceps with amnion pulled out through opening in the chorioallantoic membrane; 4 -- Pasteur pipette; 5 - amniotic sac with embryo inside egg; 6 - yolk.

For the purpose of obtaining material from the amniotic cavity its wall is exteriorized, as has been described for the infection. The infected amniotic fluid is aspirated with a pipette. The virus-containing fluid is usually turbid; its quantity varies from several drops to one cubic centimeter.

The different methods of infection described above make it possible to modify the method according to the desire of the experimenter and to utilize it for the purpose of infecting any organs of the developing embryo.

Using the method of chorioallantoic membrane infection as a basis, it is easy to apply it to the injection of material into the allantoic vein. After exposing the chorioallantoic membrane over the large vessel the material is injected into the blood stream with a fine needle or with a drawn-out Pasteur pipette.

Utilizing the technique of exteriorization of the amniotic sac it is possible to inject material into any organ of the embryo. Here, the body of the embryo is fixed with a flat-blade forceps in the left hand, and the material is injected with the right hand. Intracerebral infection is accomplished by this method.

Cultivation of influenza virus in deembryonated eggs is carried out in certain special cases. The technique of this method consists of the following (Bernkopf, 1949). A broad opening is cut out of the shell opposite the air sac, the shell membrane is incised, and the body of the embryo with its internal membranes is extracted from the egg; the chorioallantoic membrane, which is washed out, is left in situ; the egg is filled with Tyrode's solution containing penicillin and streptomycin (100 units per cubic centimeter of fluid). After the injection of the virus into the egg the opening is covered with a rubber or glass cover which is fastened with paraffin. For this method embryos 13-15 days of age are used; the incubation period after the infection depends on the problems of the experiment.

Cultivation of the influenza virus is also possible in a dead chick embryo, where multiplication of the virus occurs on the strength of the surviving cells (Lahelle and Horsfall, 1949).

For the prolonged preservation of the viability of the influenza virus in the material from the infected embryo use is made of membranes (chorioallantoic and amniotic) as well as organs (lungs and trachea). This material is extracted from the egg into a separate test-tube if the hemagglutination reaction performed by the droplet route indicates the presence of virus in the allantoic or amniotic fluid of the given embryo; the material of these organs is used for serial passages even in case the reaction in the corresponding fluids is negative.

Since multiplication of the virus occurs in the tissues of the membranes or respiratory organs of the embryo it is specifically there that the most active virus is contained, still bound to the susceptible cells.

A suspension of these tissues and of allantoic or amniotic fluid showing an active hemagglutination action is the best material for preserving the virus in a desiccated state (vacuum with freezing).

15. Method of Tissue Cultures Used in Virology

Tissue cultures, or explants, customarily means, in the broad sense, pieces of tissue or a group of cells placed in a medium of suitable composition under sterile conditions which makes it possible for the cells to multiply or survive a certain time outside the body.

The growth and development of explants have only an apparent similarity to the regeneration processes in the body. The regenerative processes which occur in the intact organism are subordinate to correlative relationships of

this organism. The tissue culture is outside of these relationships; therefore, the growth and development of tissues outside the body depend chiefly on two factors: the properties of the cells and the tissues and the composition of the nutrient medium of the cultures.

For the purpose of cultivation of influenza viruses use may be made of various methods of tissue cultivation outside the body.

The Maximow Method (1916). The Maximow method accomplishes tissue cultivation in a two-layered medium in a drop. Pieces of tissue one to three millimeters in section are placed in a mixture of blood plasma and embryonic extract on small mica plates or on small and thin cover glasses. The small mica plates or cover glasses are attached to a mica plate of larger size by a drop of Ringer's solution. A small droplet of liquid nutrient mixture consisting of blood serum, embryonic extract and Tyrode's solution is applied over the solid phase. The large mica plate is inverted over a pocket in a glass slide and hermetically sealed with a layer of paraffin along its edges. Usually after two or three days the chambers are opened up, the cover glasses containing the culture are washed with Ringer's or Tyrode's fluid, and then feeding is carried out, that is, a fresh drop of nutrient fluid is applied to the surface of the solid phase. The passage is accomplished through several similar feedings. The growth zone with this method of cultivation reaches a large size. The culture containing the large growth zone is excised with a razor or sharp knife, cut into two or more parts, which, after rinsing in Tyrode's or Ringer's fluid, are transferred to a new nutritive medium.

The Carrel Method (Smirnova, 1933). Pieces of tissues are placed on a large mica plate or cover glass in a mixture containing blood plasma and embryonic extract. After coagulation of the mixture the plate is inverted over a pocket in a glass slide of appropriate diameter and is hermetically sealed with paraffin.

With this method of tissue cultivation no feeding can be accomplished, because the mica plate is infected from the outside. Since no feeding is given the passages have to be made very frequently.

The advantage of the Maximow Method over the Carrel Method consists in the fact that the times of tissue cultivation without passages are prolonged because of the administration of food.

The Method of Cultivation in Carrel Flasks (Timofeyevskiy, 1947). A small quantity of blood plasma is introduced through the neck of the flask. Several pieces of tis-

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sue are inserted into the blood plasma before coagulation of it, and by means of a needle or uniform shaking they are distributed at equal distances from one another. Embryonic extract is added for the purpose of coagulating the plasma. After coagulation of the solid phase the liquid phase is introduced, which usually consists of diluted embryonic extract, Tyrode's solution and blood serum.

Depending on the rate of growth of the cultures, the liquid phase is replaced after 3-15 days following a preliminary irrigation with Tyrode's fluid, and the passage of the cultures is accomplished after 6-30 days, and sometimes even less frequently.

In Timofeyevskiy's laboratory an exceptionally convenient method is used for prolonged cultivation of the tissue in Carrel flasks: the tissue is explanted into Carrel flasks on small pieces of mica which have been introduced into the flask beforehand. This facilitates the accomplishment of the passage and histologic treatment of the cultures.

For tissue passages into a new nutrient medium the neck of the flask is flamed, the liquid phase is aspirated, the solid phase around the mica is separated off with a spatula or Graefe knife, raised up with a straight-limbed eye forceps, extracted from the flask, and dropped into Tyrode's solution. Then a section is made through the middle of the piece, and by means of four subsequent straight sections approximately one-third of the peripheral portion of the growth zone is cut off. During passage, well-developed cultures need to be divided into two or four parts in order to avoid excessive thickening of the central portion. After washing, each piece of tissue is explanted, as usual, in the form of a separate culture.

Cultivation of Tissue in Roller-Tubes. Gey (1933) suggested cultivating tissue in rotating test-tubes. A large quantity of tissue can be planted in such test-tubes and cultivated in a two-layered medium.

The inner surface of a test-tube with a narrow neck is covered with a fine solid phase layer in which are included pieces of the material to be cultivated. The liquid phase, consisting of diluted serum containing the embryonic extract, is poured over it, and a sterile gas mixture -- carbogen [mixture of carbon dioxide and oxygen] -- is introduced. The test-tubes are put into a thermostat contained in a special apparatus and given a rotary motion at a rate of four to five revolutions per hour. This method provides good nutrition of the growing tissue because of the continuous movement of the fluid, which eliminates the accumulation of metabolic products at the growth areas of

the tissue.

Cultivation of Tissue in a Continuous Current of Liquid Nutrient Medium. This method has been used by Haan (1929), Sokolov (1939), and other authors.

This method of cultivation creates the most favorable conditions for the growth of the tissue, but it requires large quantities of nutrient medium and a complex apparatus.

Method of Surviving Tissues. The method of virus cultivation in live but not multiplying cells has become the most widespread in virology. Thus, the Maitlands (Maitland and Maitland, 1928) successfully cultivated viruses in Erlenmeyer flasks in a special medium. The difference of this medium from that used in classic tissue cultures consisted of the fact that it did not contain plasma, that is, the mechanical factor which is responsible for cell proliferation. Because of the absence of the necessary mechanical support the tissue elements were deprived of the possibility of multiplication.

Many investigators have successfully cultivated viruses in surviving tissues, somewhat altering the composition of the nutrient medium.

Rivers (1930) and his associates simplified the method by removing serum from the liquid portion of the medium. The authors passed the vaccinia virus through scores of generations outside the organism using a medium consisting of chick embryo tissues suspended in Tyrode's solution, while Magill and Francis (1936) used this method for the cultivation of influenza viruses.

The Fadeyeva Method. This method represents the application of the method of cultivating human embryonic lung tissue in Carrel flasks in a two-layered medium for cultivating influenza viruses. The original method of Fadeyeva (1952) has been simplified and perfected (Zakstel'skaya and Ritova, 1935; Ritova and Sakstel'skaya, 1954). Since this method has become widespread for obtaining vaccine strains of the influenza virus a more detailed description of it is being presented.

In Fig. 31 the appearance of the work table and the necessary appurtenances are shown for cultivation of the tissues.

Human embryonic tissues may be obtained from bynecological departments of lying-in-homes in the form of fresh sterile scrapings of the uterine cavity following artificial abortions for medical or other reasons. A careful study needs to be made of the case histories of women being subjected to abortions in order to eliminate the possibility of introduction of such infections as syphilis,

tuberculosis, epidemic hepatitis and others into the tissue cultures. The uterine cavity scrapings obtained during the first few hours of abrasion are processed in a sterile cubicle with the aim of finding the parts of the embryo, which has usually been destroyed. In scrapings of a uterus which has been 12-15 weeks pregnant it is possible to find all the lobes of the lungs having a total weight of up to 300 milligrams. The lungs of the embryo may be preserved in Tyrode's fluid at a temperature of +4° for 48 hours.

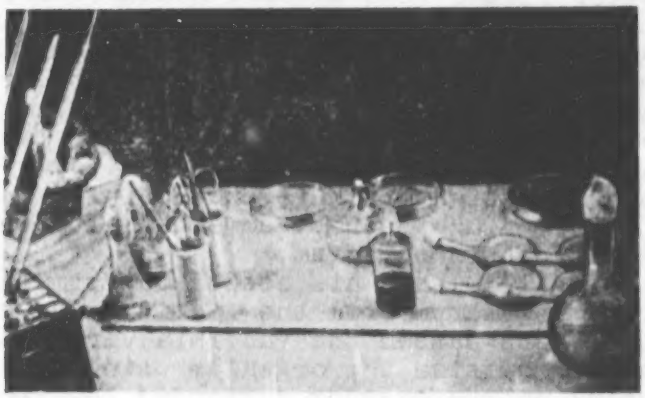

Fig. 31. Work Table in a Cubicle and Necessary Appurtenances for Tissue Cultures (Fadeyeva, 1952).

Pulmonary tissue which has been minced into pieces (Fig. 32) measuring one to two millimeters in diameter with a safety razor is washed in Tyrode's fluid for 30-40 minutes, replacing this fluid three times in order to rule out completely the possibility of neutralization of the influenza virus by tissue fluid which in the majority of human embryos possesses a virus-neutralizing activity. When the pieces of tissue are completely washed the Tyrode's fluid is aspirated, and the virus is added to the finely-divided tissues. Contact of the virus with the tissue is continued for 20 minutes, best in the cold, after which the tissue is transferred to Carrel flasks measuring 35-50 millimeters in diameter by means of a Pasteur pipette. Sixty to 80 pieces of the tissue are introduced into each cup. For the first infection of the tissue cultures 1000 LD_{50} of the influenza virus strain which has been titrated against developing chick embryos, is usually used for the 60-80

pieces of tissue.

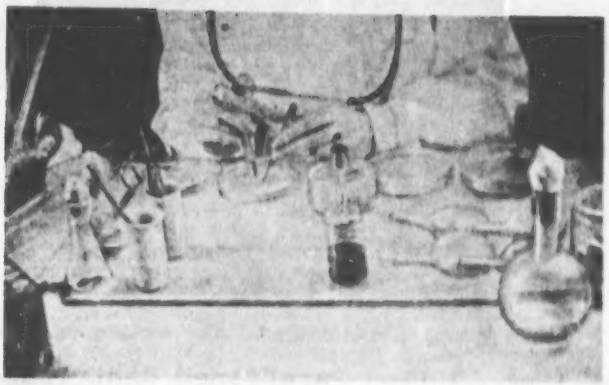

Fig. 32. Cutting the Tissue with a Safety Razor Blade (Fadeyeva, 1952).

Preparation of Ingredients of Nutrient Medium

The Tyrode fluid receipe is as follows: sodium chloride, eight grams; potassium chloride, 0.2 gram; calcium chloride, 0.2 gram; magnesium chloride, 0.1 gram; sodium monophosphate, 0.05 gram; sodium bicarbonate, one gram; glucose, one gram; doubly distilled water, 1,000 cubic centimeters; the pH of such a fluid is equal to 7.6. The fluid is sterilized by filtration through asbestos Seitz filters.

Plasma. For the purpose of cultivating the influenza virus in explants of human embryonic lung tissue use may be made of sterile human and chick plasma (Fig. 33) with heparin. The heparin solution is prepared in physiological saline solution, autoclaved and added according to the calculation of one cubic centimeter of the solution (1:500) per 20 cubic centimeters of blood. The sterile plasma may be kept in paraffinated test-tubes at 4° for two to three months.

Serum. Inactivated chick or human serum may be used for making tissue cultures. The sera are inactivated in a water bath at 56° for 30-40 minutes. All the sera used for making tissue cultures should be without virus-neutralizing activity with respect to the influenza virus. The sera may be preserved at 4° and used for two years.

33a

Fig. 33. Filling Chilled Paraffined Centrifuge Tubes with Fresh Blood Taken From a Chick's Heart for the Purpose of Preparing the Plasma.

Embryonic Extract. Usually, the extract is prepared from the tissues of seven-day chick embryos. Washed pieces of the embryo deprived of brain tissue and eye pigment are ground up to a homogeneous consistency with a glass pestle in a centrifuge tube; Tyrode's fluid is added for the purpose of obtaining a 30-percent suspension. After centrifugation for 10-15 minutes at 2500 revolutions per minute the clarified supernatant fluid -- the embryonic extract -- is aspirated and preserved at 4° for 7 to 10 days.

All the ingredients of the nutrient medium listed above are checked for sterility before making the cultures.

After the pieces of tissue infected with the influenza virus are transferred to a Carrel flask 0.3 cubic centimeter of heparinized chick plasma, 0.5 cubic centimeter of the nutrient mixture consisting of one part chick or human serum and three parts Tyrode's fluid are added to them.

For the purpose of coagulating the plasma 0.2 cubic centimeter of 30 percent embryonic extract is added. In order to distribute the pieces of tissue uniformly the flasks need to be uniformly shaken. After coagulation of the plasma in the Carrel flask the liquid phase, consisting 1.5 cubic centimeter of the nutrient mixture, is added in a sterile manner. Then, the cups are incubated in the dark at 36-37° for 48-72 hours.

Quantitative determination of influenza virus accumulation in the tissue cultures is accomplished by titration of the supernatant fluid against developing 11-day chick

embryos, comparing the maximum virus titer in the tissues before the onset of incubation (control Carrel flasks) with the titer after incubation of the cultures (experimental Carrel flasks).

The virus material for the passages is prepared by means of mincing the tissues extracted from the Carrel flasks after 48-72 hours of incubation in a sterile cubicle, observing surgical asepsis. First of all, the contents of the Carrel flasks are transferred to a centrifuge tube. After centrifugation in beakers containing ice, the culture fluid of the entire mixture is aspirated. The precipitate is carefully ground up in a porcelain mortar, and Tyrode's fluid is added to the ground-up precipitate according to the calculation of one cubic centimeter of fluid per 60-80 ground-up pieces of tissue extracted from a single Carrel flask. The suspension prepared in this way is transferred to a centrifuge tube and centrifuged until it is half clear. It is usually arbitrarily considered that in such supernatant fluid the tissue-extracted virus is contained in a dilution of 10^{-1}.

The supernatant fluid is utilized for the quantitative determination of the virus in tissue cultures and for the purpose of infecting subcultures.

The infection of subcultures is carried on by the usual method according to the calculation of 0.1-0.2 cubic centimeter of the supernatant fluid per 60-80 pieces of washed-out embryonic tissue.

The use of antibiotics. The influenza virus does not accumulate in tissue cultures which have been contaminated with bacteria. Therefore, all the manipulations in making tissue cultures and in preparing material for passages should be carried out in a cubicle, boserving all the aseptic requirements. The cubicle is carefully washed out with three percent carbolic acid and irradiated with an ultra violet lamp. Gowns, neckerchiefs and sterile masks are sterilized in an autoclave. Before work, the hands are carefully washed with soap and then treated with alcohol. The glass-ware for making the cultures is carefully prepared. The Carrel flasks, Maximow chambers, Petri dishes, test-tubes, centrifuge tubes and pipettes are washed with potassium dichromate, and then the vessels are washed out in running water for 24 hours and dried in a desiccator. The instruments for treatment of the tissues should consist of two surgical and two anatomical eye forceps, blunt-tipped scissors, a platinum needle and two Pean hemostatic forceps for the purpose of fixation of the safety-razor blades. Before work, the instruments are sterilized by boiling.

In making the tissue cultures use may be made of

penicillin and streptomycin, which are used only in cases where there is reason to suspect the possibility of bacterial contamination; 50 units of penicillin and 50 units of streptomycin in two cubic centimeters of the nutrient mixture assure a complete sterilization of the cultures and have no influence on the quantitative accumulation of the virus in them.

For certain laboratory investigations use is made of the cultivation of influenza virus strains not only in explants of human embryonic lung tissue but also in mouse embryonic lung tissue. For the purpose of making cultures from mouse embryonic tissue gravid mice weighing 18-20 grams are sacrificed. The embryos, numbering from four to eight and each weighing 700-900 milligrams, are extracted from the abdominal cavity in a sterile manner and transferred to Petri dishes containing physiological solution. Then, the trachea and lungs are rapidly extracted and washed out in Ringer's solution and cut into pieces measuring one to two millimeters in diameter and with even edges with a safety-razor blade. The minced tissue is carefully washed in Tyrode's fluid until it is free of formed blood elements. Then, the tissue is infected with influenza virus, as has been described above, and transferred to Carrel flasks, to which the nutrient medium is added. The nutrient medium usually consists of two phases. The solid phase contains 0.5-0.7 cubic centimeter of the nutrient mixture consisting of one part mouse serum and three parts Tyrode's fluid, 0.1 cubic centimeter of heparinized chick or mouse plasma and 0.2 cubic centimeter of 30 percent mouse embryonic extract. Immediately after coagulation the liquid nutrient phase, consisting of 1.5 cubic centimeter of the nutrient mixture, is added to these cups. The Carrel flasks, as usual, are incubated at a temperature of 36-37°.

The influenza virus shows a good growth in the explants of the chick embryonic tissues.

In using the method described microscopic examination of the tissue cultures makes it possible to detect the characteristic pictures of its growth (Fadeyeva, 1952). After 24 hours of incubation a slight migration of formed blood elements is noted into the plasma surrounding the pieces of tissue. After 48 hours a sparse growth in the form of individual strands is found in half of the pieces of tissue. After 72 hours, the growth zones are found in the majority of pieces of tissue (Fig. 34, 35). On the fourth day, the growth zone has increased considerably in size (Fig. 36), and desquamated areas of the epithelial membrane appear (Fig. 37). Afterwards, a considerable

growth of fibroblasts (Fig. 38) is observed, and a degeneration of the epithelial cells occurs (Fig. 39).

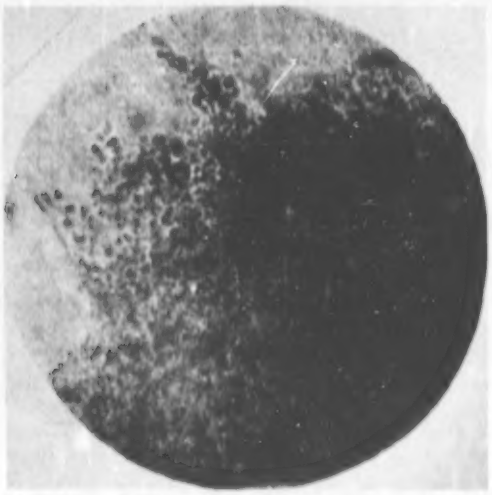

Fig. 34. Three-Day Culture of Lung Tissue of Seven-Month Human Fetus. Epithelial Membrane. Total Preparation. Iron Hematoxylin According to the Heidenhain Method (Fadeyeva, 1952).

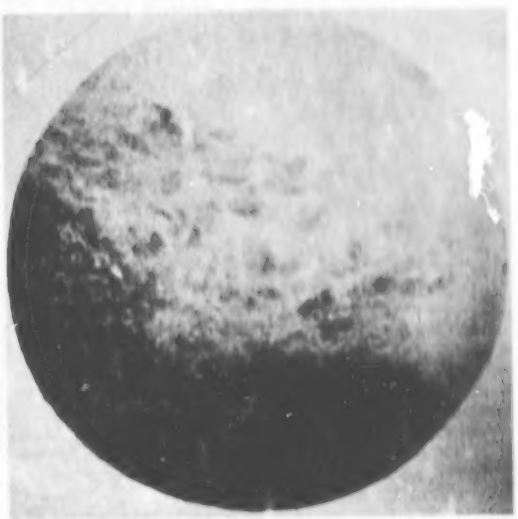

Fig. 35. The Same Preparation as in Fig. 34 with Greater Magnification. The Growth Zone of the Epithelial Cells is Seen (Fadeyeva, 1952).

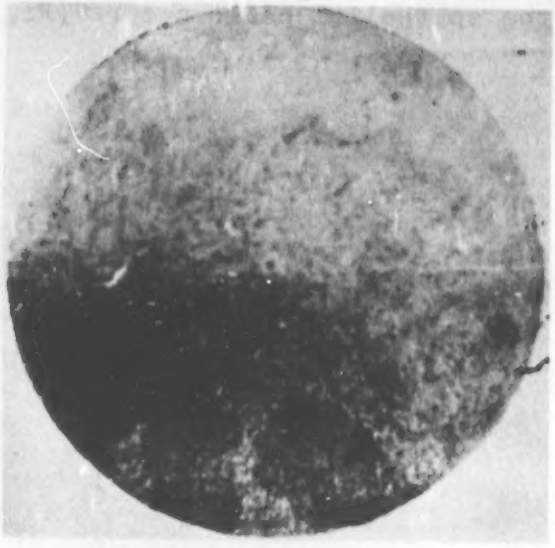

Fig. 36. Four-Day Culture of Lung Tissue of Four-Month Human Embryo. Total Preparation. Iron Hematoxylin According to the Heidenhain Method (Fadeyeva, 1952).

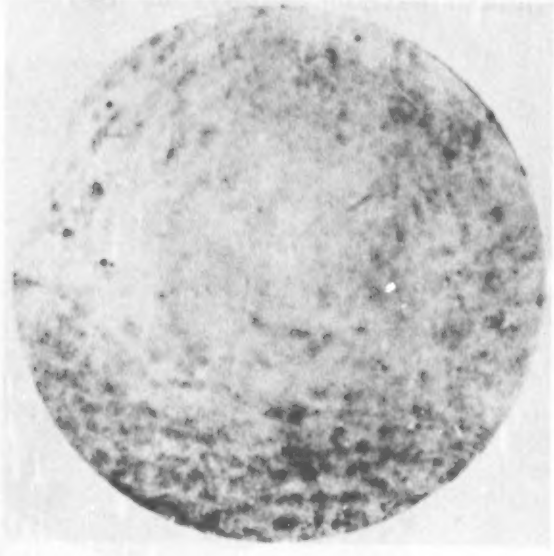

Fig. 37. Four-Day Culture of Seven-Month Human Fetus. Desquamated Area of Epithelial Membrane. Iron Hematoxylin According to the Heidenhain Method (Fadeyeva, 1952).

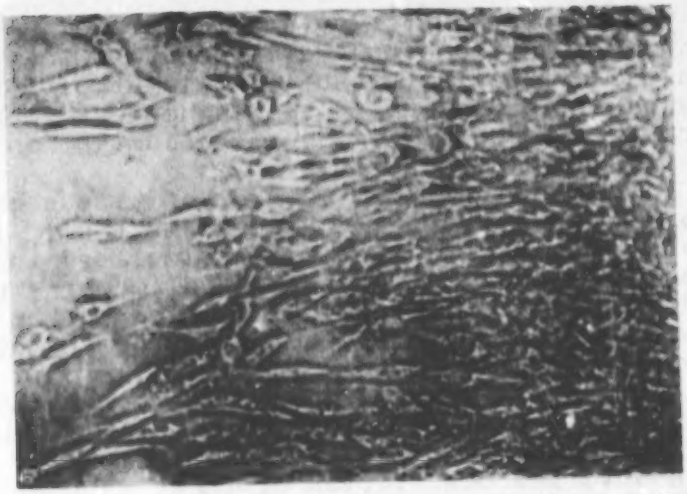

Fig. 38. Six-Day Culture of Lung Tissue of 14-Day Human Embryo. Considerable Growth of Fibroblasts (Fadeyeva, 1952).

Fig. 39. Section of Culture Shown in Fig. 38. Degeneration of Epithelial Cells (Fadeyeva, 1952).

The optimum incubation periods of the tissue cultures after they are infected with influenza viruses are 48-72 hours for the A and A-prime viruses and 72-96 hours for the B viruses. The optimum temperature for development

of the viruses is 36-37° for A and A-prime; 35-36°, for B. Under these conditions the development of the viruses occurs in the epithelial tissue. At later periods, when the growth of epithelium is slowed down by fibroblasts and degenerative processes begin, the accumulation of virus in the tissue culture stops.

In recent years new methods of tissue culture have been worked out for growing viruses in them. These investigations have been particularly stimulated in connection with the development of methods of diagnosing poliomyelitis and other viruses as well as in connection with the development of mass production methods for the poliomyelitis vaccine.

It must be supposed that these new methods, particularly the method of monolayer cultures, will be used also for the cultivation of influenza viruses.

A detailed description of these methods may be found in Levi's monograph (1957).

16. Passages in Laboratory Animals

White mice, ferrets or white rats may be used for the isolation and passage of influenza viruses. Other laboratory animals are less often used and usually for special research. Since the methods of infection of laboratory animals have been described in textbooks on virological technique (Shubladze and Gaydamovich, 1949), only a brief description of the methods of animal passage which are most frequently used in the study of influenze viruses will be presented here.

Passages in Mice. As the star ing material use is made of a suspension of mouse lungs infected with influenza virus or allantoic virus cultures. Formerly, the mice were used also for direct isolation of the virus from a washing. This material was used in the untreated form or treated in the same way as for infection of chick embryos.

The animals are first anesthetized, for which purpose they are placed in a glass jar, pieces of cotton moistened with ether are thrown in, and the cover is applied. After one or two minutes, the mice become inactive. Mice in a state of anesthesia are taken out of the jar, and two to three small drops of fluid containing the virus (Fig. 40) are introduced into each nasal opening by means of a Pasteur pipette with a finely drawn-out tip. When the anesthesia has been properly given, which is acquired by practice, the drops of fluid enter rapidly into the nasal openings. If the anesthesia is too deep the fluid does not enter the respiratory passages well, and if it is insuffi-

cient the fluid is ejected from the nares.

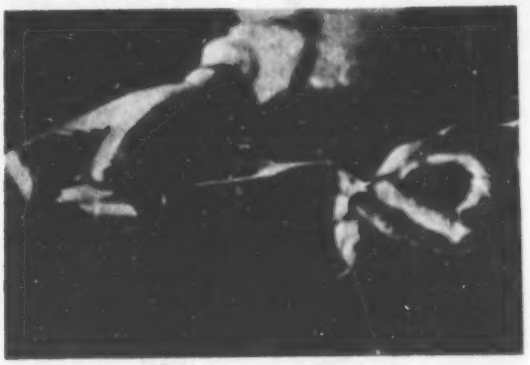

Fig. 40. Technique of Intranasal Infection of Mice With Influenza Virus (Shubladze and Gaydamovich, 1949).

The animals become ill after a two-three-day incubation period, which may be shorter (after infection with highly virulent adapted strains). After infection with unadapted strains an asymptomatic infection may develop. The principal signs of the disease are sluggishness, refusal of food, and, with the development of pneumonia, rapid respiration with a crepitant sound. After infection with virulent strains the mice die after one or two days. At autopsy a hemorrhagic pneumonia (characteristic dark red color of the affected areas of the lungs) is found with involvement of the lungs, complete or partial.

For the purpose of further passages use is made of the lung suspensions. The latter are extracted, ground up in a sterile mortar with sand. The suspension is diluted 10 times with physiological solution, and centrifuged. The supernatant fluid is used for the infection.

For the purpose of solving certain problems the method of infecting the mice in a chamber sprayed with the virus is used. A convenient modification of this method has been proposed by Vashkov and Serebryakova (1951); they designed a simple apparatus which may be prepared in any laboratory. The mice are placed in a chamber with a capacity of 250 liters. Spraying of the virus is accomplished by means of a glass jet-sprayer (Fig. 41) which is inserted into an opening in the chamber. A vacuum cleaner or compressor having a positive pressure of 0.5-1 atmosphere may be used as a blower. Four-tenths to 0.5 cubic centi-

meter of the virus-containing suspension is used for a three-minute spraying.

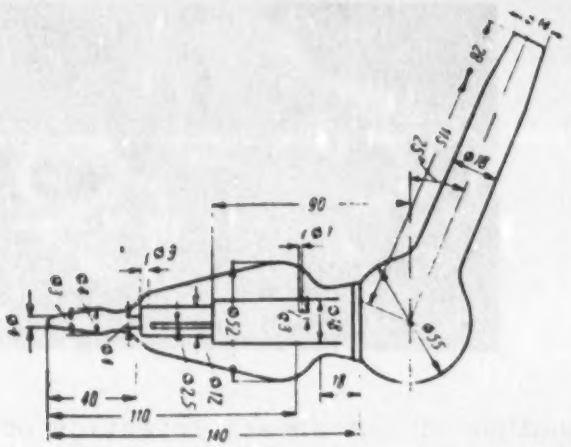

Fig. 41. Jet-Sprayer. The Internal Dimensions are Indicated in Millimeters. The Glass of the Entire Apparatus is One Millimeter Thick (Vashkov and Serebryakova, 1951).

Passages in Ferrets. The sputum or nasopharyngeal washings of influenza patients are used as the starting material for isolation of the virus. At first (Smith, Andrewes, Laidlaw, 1933), washings were used in the filtered form (through bacterial filters) or in the unfiltered form, since the bacterial microflora of the human nasopharynx is usually not pathogenic for ferrets. At the present time, one of themodifications of antibiotic treatment of the material is used with concentration of the virus on erythrocytes, just as for the infection of chick embryos. The animals are first anesthetized with ether in a jar or by means of a mask, after which the material is introduced into the nasal passages with a pipette or by means of spraying with an inhalator.

The animals become sick after a two-day incubation period. The principal signs of the disease are fever, the occurrence of nasal excretions, conjunctivitis, and refusal of food. The disease is not lethal. At autopsy, inflammatory signs are found in the trachea and bronchi; the lungs are usually not involved. For the purpose of further passages use is made of a scraping from the air passages which is suspended in physiological solution and introduced into the nasal passages of fresh animals. In view of the contagiousness of influenza in ferrets the virus passages

may be made by means of putting healthy animals into contact with the sick ones; the latter are contagious for five days after the onset of the disease.

In view of the contagiousness of influenza for them, in working with ferrets the animals should be kept in strictly isolated cages with exhaust ventilation. Keeping sick and healthy animals in the same room should not be permitted, and the healthy animals should not be kept in the same room with animals infected with various strains of influenza virus. Incidentally, this rule should be observed also in working with mice and other animals infected by the respiratory passages.

Passages in Other Animals. Of the other animals use is made of white rats, guinea pigs, rabbits, hamsters, susliks, hedgehogs, and monkeys for the passage of the influenza virus. These animals are not very susceptible or are resistant to influenza, and therefore they are used only for special investigations.

Passages in rats are accomplished by means of intranasal or intratracheal introduction of the virus. The rats have been used both for isolation of the virus and for subsequent passages. The passages of the influenza virus in rats were first successfully accomplished by Agapov (1936). The technique of intranasal infection of the rats is the same as for the mice. Intratracheal infection is accomplished either with a syringe and blunted needle through the mouth or by means of a puncture of the trachea from the outside; in both cases the animals should be anesthetized first. For the passages use is made of lung tissue or a washing of the contents of the air passages in physiological solution.

The methods of infection and subsequent passage of the virus in guinea pigs, rabbits, hamsters, susliks, hedgehogs and other small laboratory animals are similar to the methods described for working on ferrets and white rats. Infection of monkeys is accomplished by means of instillation of the material into the nose with a pipette or by means of spraying it with an inhaler. For further passages use may be made of washings from the nasopharynx obtained by means of putting cotton tampons into the nasal passages.

Special Methods of Studying Influenza Viruses in Laboratory Animals. For the purpose of solving special problems (pathogenesis and immunity, toxic properties of the virus, study of variability, etc.) use is made of certain other methods of infecting the animals and subsequent virus passages.

Subcutaneous, intracutaneous, intravenous, intraperitoneal and other methods of introducing the influenza virus

which are used in the study of pathogenesis and immunity are carried out according to the method which is described below. Methods of passages through tumors and methods of determining the toxic properties of the influenza viruses require special analysis.

Passages through tumors are carried out according to a method developed by Shen (1949) which has been used by Vanag (1950) for the study of influenza viruses.

For the purpose of cultivating influenza viruses in tumors use is made of the method of indirect infection of the tumor substrate. The passages of influenza virus are carried out on mice into which the tumors have been grafted 9 to 10 days before the injection of the influenza virus. As a tumor substrate use is made of the Crocker mouse sarcoma strain, the mouse carcinoma C63 strain and others. The grafting of the tumors is accomplished subcutaneously. For the purpose of the inoculations use is made of a bacteriologically sterile tumor from which glistening pieces without foci of necrosis are excised and cut into small pieces with scissors. Several pieces are placed in a trochar, which is injected into mice under the skin of the back. The pieces are expelled by a movement of the plunger, after which the trochar is carefully removed. The entire course of movement of the trochar is controlled with the thumb and index fingers of the left hand.

Mice which have been infected by the tumors are infected with influenza virus intranasally. For the purpose of the infection use is made of the ordinary influenza virus dose. After an intranasal infection of the mouse the influenza virus circulates in the blood stream for the first two days and seeds the tumor substrate. During the first two or three days after the infection the influenza virus accumulates not only in the lungs but also in the tumors.

The dynamics of accumulation of the influenza virus in the tumor substrate and in the lungs are determined in parallel according to the data of the hemagglutination reaction and in experiments of biological titration against healthy mice and chick embryos.

17. Methods of Determining the Toxic Properties of the Influenza Virus

Methods of studying the toxic properties of the influenza virus have been worked out by Rickard and Francis (1938), Hale, McKee (1945), Evans and Rickard (1945) and Zakstel'skaya (1951, 1953), who, in addition to the methods previously proposed, was the first to use (Zakstel'skaya

and Tarusov, 1950) the tissue-constriction reaction according to the Tarusov method (Tarusov and Khayt, 1944) for the determination of the toxic properties of influenza viruses.

For the purpose of determining thetoxic properties of the influenza viruses use is made of the following methods: a) intravenous injection of material into mice, rabbits, guinea pigs, and other animals; b) intraperitoneal injection of material into mice; c) injection of the material into mouse brain; d) injection of the material into the anterior chamber of the eye of guinea pigs and rabbits; e) study of the tissue constriction according to the Tarusov method. Other methods of determining the toxic properties of the influenza viruses are possible also (intracutaneous tests on various laboratory animals, injection of massive doses of the virus into the lungs of mice, utilization of chick embryos, etc.

For the purpose of testing for toxicity use may be made of allantoic cultures and lung suspensions of the influenza virus.

Preparation of the Allantoic Cultures. For the purpose of testing for toxicity of various viruses cultured on the chick embryo it is recommended that several (two or three) successive passages be made by the allantoic route, utilizing a much diluted virus (dilution of 10^{-5}-10^{-6} of the whole allantoic culture) for the infection. Usually, 10-11-day embryos are infected which are then incubated at 37° for 48-72 hours. After chilling until complete stasis of blood occurs in the embryonic blood vessels the infected embryos are dissected, observing strict asepsis. The allantoic fluid is aspirated from each embryo into seperate test-tubes.

Material from each test-tube is plated out onto nutrient media (meat-peptone agar slant and meat-peptone bouillon), and a small quantity of the fluid is also taken out for the determination of the concentration of virus. This test is accomplished by the hemagglutination method in increasing serial dilutions.

The test-tubes containing allantoic fluid are kept at 4° for 48 hours, after which the test-tubes are taken out, free of bacterial flora and containing virus in a dilution of no less than 1:160 as measured by the hemagglutination reaction.

The contents of the test-tubes are combined in a sterile manner into a single flask which is kept in a refrigerator at 4°. In the absence of any temperature fluctuations such as sterile allantoic fluid can be utilized for two or three weeks as a preparation which exerts a toxic effect.

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Preparation of the Lung Suspension. Mice weighing 8 to 10 grams are infected under ether anesthesia using a suspension of the passaged virus in a dilution of 1:100 and 1:1000. After 48 or 72 hours the animals are sacrificed, their lungs are extracted in a sterile manner, and each one is placed in an individual dry, sterile centrifuge tube. In addition, a piece of lung is dropped into bouillon for the purpose of checking it for the growth of bacterial flora.

The test-tubes containing lungs are kept at 4° or in a special refrigerator at -20° or -40°. After 48 hours, the lungs which are free of bacterial flora are combined, ground up in a mortar and suspended in sterile physiological solution according to the calculation of two cubic centimeters of physiological solution for one lung. The suspension obtained is centrifuged 20 minutes at 3,000 revolutions a minute. After this, the transparent supernatant layer is aspirated and tested for toxicity on the same day. The concentration of virus in the suspension is determined by the hemagglutination reaction. The prepared suspension is considered to be a dilution of 1:20. For the purpose of testing for toxicity suspensions the hemagglutinating titer of which is no less than 1:640 or 1:1280 are suitable.

When the material is injected intravenously into mice the lung suspension is used in dilutions of 1:80 in a dose of 0.25 cubic centimeter or over to avoid nonspecific protein reactions.

For the purpose of testing for toxicity natural influenza virus suspensions from both allantoic fluid and lung tissue can be subjected to a preliminary concentration. For this purpose supercentrifugation of the cultures in the cold for 1 to 1.5 hours at 15,000-16,000 revolutions per minute with subsequent suspension of the precipitate in a smaller quantity of physiological solution is recommended. The method of chick-erythrocyte adsorption with subsequent elution in a smaller quantity of physiological solution can also be used.

Method of Injecting the Preparations Being Tested into Animals. The capacity of the preparations for exerting a general toxic-lethal effect is tested by means of injecting the material into animals intravenously, intraperitoneally and intracerebrally. Of the animals the best suited for this purpose are mice weighing 8 to 10 grams; however, for experimental purposes use is made of other laboratory animals: rats, rabbits, and guinea pigs.

a) Intravenous Injection. The tail of a mouse is heated in warm water, and the preparation being tested is

injected slowly into the distended caudal vein through a fine syringe needle. The allantoic fluid is injected in a volume of one cubic centimeter; lung suspension, in a volume of 0.25 cubic centimeter.

b) Injection into the Peritoneal Cavity. The mouse is fixed in a vertical position head down; the lower part of the abdomen is smeared on the left side with alcohol and iodine, a syringe needle puncture is made through the abdominal wall from above down, and 0.5 cubic centimeter of the material being tested is injected into the cavity.

c) Intracerebral Injection. The mouse is held by the tail and the skin of the occiput by the left hand. The area of skin over the left eye is copiously smeared with tincture of iodine and alcohol. A puncture of the cranial bone is made in the supraorbital area with a fine, slightly blunted needle. The needle is injected to a depth of 1.5 millimeters, and 0.03 cubic centimeter of the preparation being tested is carefully introduced into the depth of the brain substance.

For the purpose of determining the titer of the general toxic-lethal effect dilutions are made from the preparation being tested in sterile physiological solution using a coefficient of two, and mice are injected by the methods listed above in the doses indicated.

The animals treated in this way are observed continuously for the first three hours for the purpose of determining traumatic complications, shock and nonspecific protein reactions. The animals which die in this time interval are eliminated from the experiment.

After this, the recording of the condition of the animals is accomplished twice a day for five days. The characteristic features of a specific toxic state are usually noted after 15-24 hours. Among them are sluggishness, progressive stupor and inactivity, and tremor of the head. Convulsive attacks are observed regularly, during which the animal usually dies. At autopsy hemorrhagic-necrotic changes in the liver, a bloody exudate in the peritoneal and pleural cavities and in the lumen of the intestines, multiple punctate hemorrhages in the intestinal mucosa, in the mesenteric glands and apex of the heart are characteristic of the specific toxic state. As a rule, the lungs are congested and show no inflammatory foci.

The dilution of the preparation being tested which causes death of half of the animals in five days is considered to be that containing a single toxic-lethal dose of the toxic substance.

Determination of the capacity for a local reactive-toxic effect in the case of the influenza virus is accomp-

lished usually by means of the injection of suspensions into the anterior chamber of the eye of rabbits or guinea pigs. The intracutaneous injection of cultures into mice may also be used for experimental purposes.

For introducing the virus into the anterior chamber of the eye the rabbit is held with its side up on the operating table. The animal is covered with sterile napkins with a free space left over the eye area.

Prior to the experiment, 0.5 cubic centimeter of two-percent novocain solution is instilled into the optic fissure, after which the eye is forcibly closed and kept in this condition for 5 to 10 minutes until tactile sensation in the cornea finally disappears. Then, the eye is carefully drawn out of the orbit, held between the thumb and index fingers, and the cornea is punctured at the limbus with a fine syringe needle. The needle is injected parallel to the iris to a depth of 1.5-2 millimeters. Through the needle more than 0.2 cubic centimeter of aqueous humor is aspirated, after which the syringe is removed and another syringe is applied to the needle with which an injection of 0.2 cubic centimeter of the preparation being tested is injected into the anterior chamber of the eye. The needle is extracted, and the eye is allowed to fall back into the orbit. When the injection is performed properly, fluid should not seep out of the opening after extraction of the needle, and the ocular tension should be a little less than normal. The animal is observed for five days. The condition of the eye and the transparency of the cornea are noted. A purplish-opalescent turbidification of the cornea, because of edema developing after 48 hours and embracing the entire or part of the area of the circle around the puncture site, is characteristic of the local toxic effect of the influenza virus. Eight to 15 days later, the edema disappears, and the transparency of the cornea is restored.

The lowest dilution of the preparation being tested capable of producing edema of a quarter of the corneal disc is considered to be the unit of the local toxic-reactive effect.

Intracutaneous Injection of Mice. The fur is shaved off on both sides of the spinal column a day before the experiment. After washing the surface with 70-percent alcohol and sterile physiological solution the skin is dried with a sterile tampon. The treated section is pulled over the index finger, and 0.05 cubic centimeters of the fluid being tested is injected into the depth of the skin with a fine needle. The border of the vesicle is ringed with a chemical pencil. The animals are observed for

five days. The presence of an infiltrate and necrosis is considered to be a local toxic-reactive effect.

Apart from these, the most widespread methods, others are also used for the purpose of determining the capacity of the influenza virus for exerting a toxic effect.

a) The injection of massive doses of the influenza virus into the lungs of mice through the nose. For this purpose use is made of ordinary undiluted allantoic fluid or a lung suspension diluted 1:5.

The death of the animal occurs in one to three days. At autopsy a hemorrhagic pneumonia is found characteristic of the experimental infection; however, this pneumonia is not observed in subsequent passages (Sugg, 1949).

b) A decrease in the blood pressure of rats after the intravenous injection of an influenza virus culture into them according to the calculation of one cubic centimeter per 100 grams of weight (Kempf, Chang, 1949).

c) The temperature reaction in rabbits after the injection of influenza virus cultures into them (Bennet, Wagner, Lequire, 1949).

d) Lymphocytopenia in rabbits after they are injected intravenously with a culture of influenza virus.

e) Disturbances in the oxygen exchange in chick embryos after infection of them with concentrated doses of the influenza virus (Blumenthal and others, 1950).

In addition to these methods of affecting laboratory animals the Tarusov method, based on the recording of the tissue-swelling curve, may be used for the detection of the toxic effect of the influenza virus.

In this method the irregular swelling of muscle tissue serves as the indicator of the toxic effect. The method of the examination consists of the following. Muscles are excised from freshly killed rats or white mice, minced by means of scissors and divided into portions each containing 700 milligrams. A single portion is introduced into a glass vessel furnished with a capillary tube, and 0.2 cubic centimeter of the material being tested and 0.5 cubic centimeter of Ringer-Locke solution are introduced into the vessel. All this is carefully mixed with a glass rod, and the glass vessel is filled to the top with Ringer-Locke solution and covered with a ground cover smeared with vaseline. In this procedure special attention is directed to making sure that after covering the glass vessel the capillary which is sealed into its cover is filled with fluid and it does not contain any bubbles. The cover is fastened on with rubber rings, and the vessel is kept in a water thermostat which maintains the temperature at 37.5° with an accuracy of 0.01°.

The measuring scale of a vertical microscope is set up at the level of the meniscus of the capillary tubes, and after six minutes and until the time the glass vessel sinks the level of the meniscus is noted on the scale. A recording is made every three minutes. In the absence of toxicity in the solution a uniform swelling of the muscle occurs, and the differences between the meniscus levels are shown by a series of decreasing figures. When there are toxic substances in the solution the swelling is interrupted by a contraction, and this is accompanied by the appearance of jumps in the figures. If the jumps are noted in a second-third-fourth interval and the numerical deviations exceed twice the magnitude of the normal difference the abnormality is considered to be two or three plus, and this material is then subjected to a further investigation for specificity. For the purpose of determining the specificity of the effect the material being tested and antiserum, diluted 1:5, are mixed in equal volumes and kept at room temperature for 20 minutes; then it is examined again for toxicity. In this case, the mixture is introduced into the glass vessel in a volume of 0.4 cubic centimeter. If during the examination of the mixture the course of the curve is no different from the control it is considered that the toxicity found was neutralized by the antiserum of this type.

18. Pathogenic Properties of the Influenza Virus for Laboratory Animals

The laboratory animals which are most susceptible to the influenza virus are ferrets and white mice as well as chick embryos. The other laboratory animals are either not very susceptible (white rats) or are resistant (rabbits), or their susceptibility has been inadequately studied (monkeys).

Speaking of the pathogenic properties of influenza viruses for laboratory animals or of the susceptibility of animals to influenzal infection it should be kept in mind that during the course of passage of the influenza virus through the animal organism it essentially changes its biological characteristics in adapting to new environmental conditions. This problem will be examined in greater detail in the chapter on the variability of influenza viruses. Here, we should like to note only that the influenza virus which is in the human body becomes pathogenic for laboratory animals during the course of adaptation to existence and to multiplication in the bodies of these animals. Therefore, in this section we shall deal, to a con-

siderable degree, with the viruses which have become adapted to the bodies of various animals.

A number of problems pertaining to the pathogenesis of experimental influenze will also be analyzed in the appropriate chapter.

Ferrets (Mustela furo). After intranasal infection the ferrets become sick after a two-day incubation period. The temperature of the animals rises to 40.5-41°; the animals become sluggish, refuse food. On the second day of the disease watery discharges occur from the nose and eyes which afterwards become thicker, mucopurulent, forming a membrane around the nasal openings. The animals have difficulty in breathing because of stoppage of the nasal passages with secretion, and rales are heard on respiration. The disease lasts five to seven days, after which the temperature drops, the signs of inflammation of the respiratory passages gradually diminish and the animal recovers. The temperature is often biphasic with a drop on the third day and a new rise on the fourth to sixth day. The disease may also proceed without any definite inflammatory processes in the respiratory tracts. In animals which have been killed at the height of the febrile period an actue inflammation is found of the nasal mucosa and of the mucosa of the nasopharynx and the bronchi (Smith, Andrewes and Laidlaw, 1938). A similar course of the infection occurs after inhalation of the infective nidus (Vieuchange, 1948).

The ferrets are not susceptible to other methods of introducing the virus. After the virus is injected subcutaneously and intraperitoneally into the bodies of animals antibodies are elaborated, and they become partially or completely immune to infection through the respiratory tracts.

White Mice. After intranasal infection with virus strains virulent for the mice the disease develops after a one to three-day incubation period. The animals become sluggish, inactive, lose appetite, develop a nasal discharge, emitting distinct sounds of crepitant nature. Death usually occurs during the first two to four days after the onset of the disease. At autopsy the lungs are of a dark red color, almost without air, with emphysematous sections. In mice which have been killed various degrees of pulmonary involvement are observed -- from small foci to total involvement of all the lobes of the lung. The incubation periods may drag on to four or five days; the time of death may be extended to 14 days (Andrewes, Laidlaw and Smith, 1934).

Mice, like ferrets, are not susceptible to other

methods of infection, and when they are injected with the virus subcutaneously and intraperitoneally they develop an immunity (which is expressed to varying degrees) to intranasal infection. However, there are indications of the possibility of producing influenza in mide with a fatal outcome when they are fed with food containing highly virulent influenza virus strains (Dreguss, 1943).

House mice (Mus musculus) are as susceptible to influenza as white mice (Shubladze, 1941).

White Rats. White rats were first suggested by Agapov (1936) as a laboratory model for studying influenza. Working with a mouse strain of human influenza virus (filtrate of suspension of mouse lungs was introduced into rats through the nasal openings), the author observed conjunctivitis, the excretion of a serous and then seromucoid secretion with an admixture of blood from the nose, sneezing, a depressed condition in the animals after 24 hours. All the signs of the disease disappeared between the 12th nd 22nd day after infection. With the passage of the virus in rats killed at the height of development of the disease an inflammation was found in the upper respiratory passages with pneumonic foci of catarrhal nature. The virus of swine influenza produced a milder disease.

With further investigation, however, many authors have shown that white rats are not very susceptible to influenza, and when they are infected intranasally they have an asymptomatic infection.

During the course of adaptation of the influenza virus to the bodies of rats it is sometimes possible to produce signs of a milder disease which ends in recovery. Involvement of the lungs is not observed or else has an inconstant character, although the virus can be found in the upper respiratory passages and in the lungs (Stuart-Harris, 1937).

Chalkina (1943) studied the susceptibility of white rats to the influenza virus in detail. After the infection of young animals intranasally with mouse virus a multiplication of the virus occurs in the lungs in the next 24-72 hours which reaches 10^4 MLD per cubic centimeter for mice. In killed animals a diffuse but not very intense involvement of the entire respiratory tract is observed; the picture of involvement occupies an intermediate position between similar pictures in mice and ferrets.

Rats are readily infected from one another through direct contact as well as through the air of a common cage. The rats have been shown to be suitable for isolation of the influenza virus from sick people, because up to 41 percent positive results have been obtained through the investigation of 149 patients (Gulamov and Chalkina,

1943). However, the method of isolation of the virus on embryos produces more effective results.

Other species of rats susceptible to influenza are Zygodontomys microtinus stellae and Proechimys cayennensis o'connell (Weir, 1944), cotton rats, Sigmodon hispidus (Taylor, 1940) as well as gray rats (Agapov, 1936, 1937; Gorbunova, 1948).

White rats are altogether insusceptible to other methods of infection, but they react constantly with the production of antibodies. This fact is utilized for the purpose of obtaining immune sera.

Hamsters. The influenza virus readily adapts to hamsters. Both the Syrian (Cricetus auratus) and European (Cricetus cricetus) hamsters are susceptible (Taylir, 1940; Taylor and Freguss, 1940). Davenport (1951), in performing serial passages of the B virus (Lee strain) intranasally in Syrian hamsters, observed death of the animals regularly on the fifth to seventh day after infection following four passages with signs of pulmonary consolidation. Thereby the concentration of the virus was increased in the animals' lungs. Lozovaya (1942) points out that hamsters are susceptible to influenza and may be used as a laboratory model. The reactivity of the animal changes depending on the time of the year. In the summer, when the animals are in an active state, the virus produces characteristic changes in the lungs beginning with the first passages. In the winter, during the hibernation period, passages of the virus are not accompanied by any visible involvement although the virus also multiplies in the lungs.

Susliks. Susliks of various species, according to the data of a number of authors, are sensitive to the influenza virus. Thus, according to Vaynberg and Kharmats, in spotted susliks (Citellus pigmaeus) after intranasal administration of the virus (PR8 strain of the A virus) the infection is transferred without any clinically diagnosible manifestations and with the presence of inconstant involvement of the lungs of the hemorrhagic bronchopneumonia type. The virus is found in the lungs by the presence of a positive hemagglutination reaction with lung suspensions. In passages from suslik to suslik the virus soon becomes exhausted (Vaynberg and Kharmats, 1949).

Mongolian susliks (Citellus mongolicus umbratus), according to the data of Tsurumi, Ogasawara, Takaki (1939), are susceptible to influenza infection.

Red susliks (Citellus rufestus) are not very susceptible to the influenza A virus. Although after intranasal infection with mouse virus pulmonary pathology develops in

them, and the presence of the virus can be proved by passages in mice, after passage in susliks the virus is rapidly depleted without causing any pulmonary pathology. No contact transmission (in experiments where fresh animals are placed in contact with infected animals) is observed (Shubladze, 1941).

In solitary experiments Taylor and Dreguss (1940) were unable to produce an influenzal infection in the European suslik (Citellus citellus).

Minks (Mustela sibirica). According to the data of Tang (1938), after intranasal infection the minks carry the infection in an asymptomatic form.

Squirrels (Sciurotamias davidianus). According to Tang (1938), squirrels are susceptible to influenza when infected through the nose; however, the infection progresses in them in an asymptomatic form.

Small Mouse-like Rodents. Of the small mouse-like rodents in the USSR lemmings (Legurus legurus)and field voles (Microtus arvalis) are particularly susceptible to the influenza virus (A). In the experiments of Shubladze (1941) all 12 lemmings infected with a 10-percent lung suspension from white mice which had died from experimental influenzal infection became sick after four days and died in the course of 24 hours. Pneumonic foci were found at autopsy. In similar experiments death of all 10 infected field voles occurred on the fourth to fifth day after infection; at autopsy pneumonic changes in the lungs were also found. The virus from the lungs of lemmings and field voles is readily passaged in the mice.

Durand (1945)investigated several species of mouse-like rodents from Tunis for their susceptibility to influenza. Gerbillus hirtipes, Dipodillus campestris and Dipodillus dodsoni proved to be highly susceptible after infection with PR8 virus. After they were infected the same changes developed in the lungs as in the white mice. In addition, it was possible to isolate influenza viruses from sick people by means of direct infection of these rodents. Meriones shawi and Jaculus jaculus proved to be less susceptible. The infection had the same course in them as in ferrets.

Hedgehogs. Hedgehogs are susceptible to influenza, in this respect resembling ferrets. After intranasal infection with the A virus (several passages in hedgehogs were studied) these animals became sick after five to seven days; they began to sneeze, and a mucopurulent secretion came out of their nasal apertures which dried in the form of crusts. On autopsy inflammatory signs were found in the upper respiratory passages (nasal cavities); on microscopic

examination necrosis and desquamation of the ciliated epithelium were found with the formation of an exudate made of polymorphonuclear leukocytes. The trachea, bronchi and lungs were usually not affected. The infection was transmitted to healthy animals which were in contact with the sick ones. The virus could be found in the nasal discharge on the sixth to seventh day after infection and it could not be found after 14 days. At this time, the sick animals stopped being contagious for the healthy animals. Specific antibodies are found in the serum of animals which recover (Stuart-Harris, 1936).

Guinea Pigs. After infection by the respiratory tracts the guinea pigs have an asymptomatic infection which is accompanied by the production of antibodies. The virus can be found during the first few days after infection only in the nose (Stuart-Harris, 1937). McIntosh and Selbie (1937) produced a temperature reaction in the guinea pigs by the injection of mouse influenza virus; however, on passage the virus was rapidly exhausted. After subcutaneous and intraperitoneal infection the virus rapidly dies out, and antibodies appear in the blood.

Woolpert and others (1938) injected the influenza virus intranasally into a 35-day guinea pig fetus through the uterus. The virus strain (PR8) was found in the blood, lungs and internal organs of the fetus as well as in the placenta, accumulating in considerable quantities but without producing any regular pathology even after 16 passages.

Baydakova and Zil'ber (1948, 1949) obtained more exact data concerning influenzal infection in guinea pigs. The authors showed the possibility of serial passage of the influenza virus in the lungs of guinea pigs with the constant finding of the virus in the lung tissue. However, even after a number of passages the virus remains nonpathogenic for guinea pigs and produces an asymptomatic infection in them.

Rabbits. Rabbits are resistant to influenza, and the virus soon dies out when injected into them by any route. McIntosh and Selbie (1937) observed slight pathology in the lungs and a temperature reaction in rabbits infected with two strains of influenza virus A; however, no such data have been obtained by other authors. Incidentally, the temperature reaction may have depended on the toxic effect of the influenza virus (Wagner and others, 1949).

Cats. The possibility of passages of influenza viruses in cats has been shown by Lozovaya (1942). The virus isolated from cats was no different from the mouse strains with respect to virulence or immunobiological properties.

Dogs. According to the data of Titova (1954), dogs are susceptible to influenza, particularly young ones. After infection of puppies less than one month of age with A virus (Shklyaver) by means of spraying it into the air the temperature rose to 39.5-40.8° after a day; the animals became sluggish and ate poorly. Copious excretions from the nose, sneezing, conjunctivitis, dry rales in the lungs, a disturbance in cardiac activity, and leukopenia appeared. In the author's experiments two out of three infected puppies died with signs of hemorrhegic pneumonia. The virus could be isolated during the disease also from an animal which later died.

Pigs. The susceptibility of pigs (young pigs) to influenza has been established by Agapov (1936, 1937), Shope and Francis (1936) and other authors.

With infection of 6-14-week-old pigs intranasally (using PR8, and WS influenza A virus) the animals have the disease in a mild form. The fever is not very well expressed, therise in temperature occurs one to two days after infection and the signs of the disease are inconstant and not very definite. In animals sacrificed on the third to fourth day after infection foci of bronchopneumonia are found; on histologic examination there is a peribronchitis and small foci of interstitial pneumonia. The disease has a more severe course when the infection is accomplished with a mixture of the influenza virus and H. influenzae suis. The virus can be passed intranasally; it is irregularly transmitted with natural contact of the animals.

The problem of the susceptibility of pigs to the influenza virus has been recently reinvestigated by Marennikova (1953) in connection with the variability of pathogenic properties of the influenza virus during the course of adaptation to the bodies of animals. After confirming previous data concerning the susceptibility of pigs to the influenza virus and making them more exact (the experiments were performed with the A-prime virus), the authors showed that the pathogenicity of influenza virus for mice depends on the degree of adaptation of it to the body of the animal. This problem will be analyzed in greater detail in the chapter on the variability of influenza viruses.

Monkeys. Monkeys have been tested repeatedly as a laboratory model for isolation of the influenza virus. In 1918, Nicolle and Lebailly (1919) produced an influenza-like disease in monkeys (Macacus rhesus, Macacus sinicus) by means of injecting them with the sputum of patients intraconjunctivally and intranasally. McIntosh and Selbie (1937) observed a temperature reaction in monkeys (Macacus

cynomolgus); however, they were unable to obtain a definite influenza picture.

According to the data of Burnet (1941), Macacus cynomolgus (or Macacus irus) monkeys are resistant to influenza by intranasal infection. However, tracheal administration of the WS strain in certain cases produced the development of a fatal infection. Saslaw, Wilson, Doan, Woolpert and Schwab (1946) performed experiments on young Macacus mulatus monkeys and did not observe any fatal infection after intranasal or intratracheal administration of the PR8 strain. With the last method of infection symptoms developed in two of four monkeys similar to signs of influenza in people, including a temperature rise and leukopenia.

Somewhat different data have been obtained by Vieuchange (1948) on chimpanzees (Anthropopithecus niger) and Macacus rhesus. After infection of them with mouse and ferret strains of influenza virus intranasally signs of general malaise, involvement of the respiratory tracts and fever resembling human influenza developed. The monkeys recovered rapidly.

In the experiments of Panthier, Cateigne and Hannoun (1949) in Cercopithecus monkeys infected with an egg strain of the A-prime (D48) virus areas of inflammation and hepatization were found in the lungs at autopsy.

Apparently the contradictory data obtained in experiments on monkeys depended on the different degrees of pathogenicity of the influenza virus for them and also on the fact that different authors worked with different species of monkeys.

Therefore, white mice are the oost suitable laboratory animals for the study of influenza. Apart from the clear-cut clinical and pathological picture of the disease these animals are available for mass breeding and are convenient because the disease in them is not very contagious for healthy mice. Therefore, white mice have been used extensively in the study of different divisions of the influenza problem.

A considerable number of investigations have also been carried out on ferrets. The other laboratory animals have been used episodically for the solution of certain problems.

The data concerning the pathogenicity of influenza viruses for laboratory animals applies chiefly to strains adapted to mice and ferrets, as has been mentioned. Various strains of influenza virus are quite different from one another in this respect. These differences amount to the following in general.

1. Freshly isolated strains are usually less pathogenic for laboratory animals and strains which have been kept for a long time in the laboratory, particularly where they have been passed through mice and ferrets.

2. Virus strains of different species (or types) are different from one another in their pathogenicity for laboratory animals. Viruses of the A type are usually more pathogenic for mice than viruses of other types. Viruses of the A-prime type are in second place in this respect, and then come the type B viruses. The type C viruses are non-pathogenic for mice and for ferrets.

3. Within the limits of the same species of the influenza virus (A, A-prime, B) various strains differ from one another in their pathogenicity for laboratory animals.

The variability of thepathogenic properties of the influenza viruses will be analyzed in greater detail in the next chapter.

19. Hemagglutinating Properties. Methods of Investigation

In 1941, Hirst (1941) and simultaneously McClelland and Hare (1941) discovered the property of influenza viruses of producing the agglutination of chick erythrocytes. In the USSR detailed investigations were carried out first by Shubladze and Solov'yev (1943).

This property of influenza viruses immediately attracted attention and became the subject of numerous investigations. It was shown that the influenza viruses agglutinate erythrocytes not only of chickens but also of many other animals; at the same time it was shown that many viruses possess hemagglutination properties. A detailed study was made of the effect of external factors on the hemagglutination reaction. A study was made of the kinetics and mechanism of the reaction and the nature of the factor producing it. It was shown that the agglutination phenomenon of erythrocytes is a good model for the study of the reaction of the virus with susceptible cells of the host organism. At the present time, an extensive literature exists on the problem of the hemagglutination phenomenon; therefore, it is expedient to present only the most essential data.

Performance of the Hemagglutination Reaction. The nature of the hemagglutination phenomenon consists in the fact that after the addition of the influenza virus to a suspension of chick erythrocytes or a suspension of erythrocytes of other animals a clumping occurs of the erythrocytes into aggregates and a rapid sedimentation of them.

If the reaction is performed in a test-tube, the erythrocytes, settling to the bottom of the test-tube, form a sediment with uneven edges, whereas in a test-tube without the virus the sediment is formed as a round disc with a smooth border. If the reaction is performed on a glass slide, the clumped erythrocytes resemble the picture of hemagglutination found in the determination of blood groups.

Many modifications of the hemagglutination reaction have been proposed by various authors; we shall mention only a few of them. The original method of Hirst and Pickels (1942) is based on a consideration of the optical density of the erythrocytes, because agglutinated erythrocytes settle faster than a suspension of them without the virus. The modification of Smorodintsev and Drobyshevskaya (1943) and Salk (1944) are based on a consideration of the erythrocyte precipitate at the bottom of the test-tube. The Shubladze and Solov'yev modification (1943) and the Nartsissov (1947) modification provide for the performance of the reaction on glass slides or on a plexiglass plate with pockets.

Various modifications of this reaction have also been described by Vieuchange (1948), Anderson and others (1946), Hare and MacKenzie (1947) and others.

A description of the principal methods is presented below.

Hirst and Pickels Method. For the purpose of the titration use is made of a photoelectric densitometer of a special type which is graduated in such a way that a definite density of the erythrocyte suspension corresponds to each division in microamperes.

A suspension of the virus in double serial dilutions in physiological solution is poured into test-tubes in a quantity of one cubic centimeter each, and then one cubic centimeter of standard (1.5 percent) suspension of erythrocytes (the standardization is performed first by means of a densitometer) is added to each test-tube; the mixture is allowed to stand at room temperature for 75 minutes, after which recordings are made by means of the densitometer.

For the purpose of titrating the antibodies the virus (allantoic fluid, diluted 12 times) in a dose of 0.5 cubic centimeter, the double serial dilutions of serum in a dose of 0.5 cubic centimeter and the standard 1.5 percent erythrocyte suspension in a dose of one cubic centimeter are mixed together. Recordings are also made by means of a desitometer.

This method, which has not been used in the Soviet Union, was afterwards discarded abroad also as a result of the complexity and possibility of substitution of it by

simpler and no less accurate methods of determining the hemagglutinins and antibodies.

Method of Smorodintsev and Drobyshevskaya. Blood is taken from the subalar vein of a chicken, mixed with two-percent solution of sodium citrate, filtered through gauze, and the erythrocytes are washed out three times by means of centrifugation in physiological solution. Physiological solution is poured onto the precipitate up to the original volume, after which a two-percent erythrocyte suspension is prepared from it. A suspension of mouse lung, allantoic fluid or other material being tested for virus content is diluted 1:10 and using a coefficient of two, and then poured into test-tubes in a dose of one cubic centimeter. An equal quantity of two percent chick erythrocyte suspension is added. Then, thetest-tubes are shaken up and left at room temperature for two hours, after which the reaction is recorded. With a positive result a friable precipitate with scalloped borders forms rapidly at the bottom of the test-tube; in the control test-tube (erythrocytes with a suspension of normal lungs or with normal allantoic fluid) the precipitate forms slowly and has the appearance of a disc with a smooth border.

For the purpose of performing the hemagglutination inhibition reaction serial dilutions of serum are prepared, with 0.5 cubic centimeter in each test-tube; 0.5 cubic centimeter of virus-containing suspension with an activity of two hemagglutinating units, that is, double the virus titer, considering the final dilution, which gives a distinct hemagglutination and one cubic centimeter of erythrocyte suspension are added to it. The reaction is also performed at room temperature and then recorded after two hours.

The Salk Method. Allantoic fluid or a suspension of mouse lungs containing the influenza virus is diluted in physiological solution in a ratio of 1:10 to 1:5020 and poured into a series of test-tubes in a quantity of 0.5 cubic centimeter each. Five-tenths of a cubic centimeter of 0.25 percent chick erythrocyte suspension is added to each test-tube. Five-tenths of a cubic centimeter of physiological solution and 0.5 cubic centimeter of an erythrocyte suspension is poured into the control test-tube. The test-tubes are shaken and left at room temperature for one and a half to two hours until sedimentation of the erythrocytes occurs. In the control test-tube the erythrocytes settle in the form of a dense disc with smooth borders. In the test series of test-tubes which contain virus a sediment of agglutinated erythrocytes is formed with uneven borders (the observation may be made with a mirror).

For the purpose of titration of the antibodies the greatest dilution of the virus is determined which produces

hemagglutination (which is taken as one unit), and for the work a dilution of virus containing two units in 0.5 cubic centimeter is prepared. Double dilutions of serum from 1:92 to 1:1024 and over (up to 1:16,000) in a volume of 0.5 cubic centimeter are poured into the test-tubes, and a standard virus suspension is added in a dose of 0.5 cubic centimeter to each test-tube; these are shaken up, and 0.5 cubic centimeter of erythrocyte suspension is poured in and allowed to stand at room temperature for one and a half to two hours. The following are controls: a virus with erythrocytes (the reaction should be positive), serum with erythrocytes (the reaction should be negative), physiological solution with erythrocytes (the reaction should be negative). The final dilution of the test serum which inhibits its hemagglutination corresponds to the antihemagglutinin titer. In Fig. 42 photographs of the pictures observed are presented. See the results in Table 12.

Table 12

Reading of the Reaction Shown in Fig. 42

Serum	Control	Serum Dilutions									Titer
		1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	1:8000	
Acute period	0	0	+	+	+	+	+				32
Convalescence	0	0	0	0	0	0	±	+	+	+	512

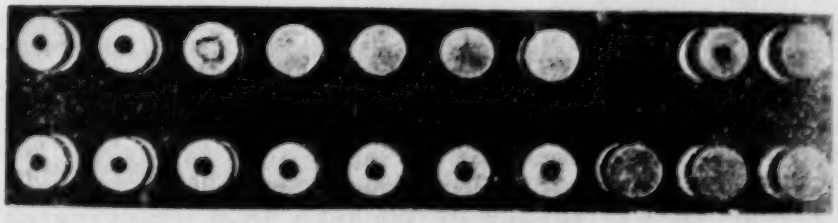

Fig. 42. Photograph of Bottom of Test-tubes During the Performance of the Hemagglutination Inhibition Reaction with Sera of Influenza Patient During

Acute Period (Upper Series) and During Convalescence (Lower Series) Showing the Typical Structure of the Erythrocyte Sediment (Salk, 1944).

The first test-tubes of both series constitute a negative control (serum + erythrocytes); the two test-tubes in the upper right are also controls: negative (physiological solution + erythrocytes) and positive (virus + erythrocytes).

The Shubladze and Solov'yev Method. Serial dilutions of the virus (1:10, 1:100, 1:1000, 1:5000, etc. in one cubic centimeter) are poured into test-tubes; these viruses are obtained from suspensions of lungs or allantoic fluid which have been clarified in the centrifuge. One cubic centimeter of washed-out chick erythrocytes, used in the form of a one-percent suspension in physiological solution, is added to the virus suspensions. The result of the reaction is read after standing for one hour at a temperature of 14°.

For the purpose of checking the specificity of the reaction 0.5 cubic centimeter of the virus is added to the test-tubes; then 0.5 cubic centimeter of hyperimmune serum and one cubic centimeter of a two-percent erythrocyte suspension are added. Depending on the experimental conditions, serial dilutions of the serum or virus are made. The control series contains a mixture of 0.5 cubic centimeter of the virus dilutions, 0.5 cubic centimeter of normal horse serum and one cubic centimeter of a two-percent erythrocyte suspension.

The authors have modified the method of the reaction by performing it on glass slides. The dilutions of the components in the test-tubes are made as has been indicated above. Then a drop of the mixture is taken from each test-tube and placed on a carefully cleaned glass slide. Agglutination occurs after three to five minutes and is read with the naked eye or by means of a lens. The reaction titers on the glass slides coincide with its titers in the test-tubes.

The Nartsissov Method. For the purpose of performing the reaction use is made of a specially prepared porcelain plate with round pockets measuring two centimeters in diameter and with a depth of six millimeters. These are arranged in six rows with 10 pockets in each. The virus-containing preparation (mouse lung suspension, allantoic fluid) in serial dilutions (1:20, 1:40, 1:80, etc.) is placed in the series of pockets in a quantity of 0.2 cubic centimeter each. Two-tenths of a cubic centimeter of two-percent chick or guinea pig erythrocyte suspension is added to each virus dilution. The fluids are mixed by

rotary movements of the bottom of a clean test-tube, beginning with the high dilutions and going down to the first dilution (the test-tube should not be wiped off); the plate is covered with a flat cuvette and placed in a thermostat at 37° for one hour, after which the results of the reaction are read. The quantity of hemagglutinin contained in 0.2 cubic centimeter of the virus suspension of the highest dilution which produces a distinct erythrocyte agglutination is taken as one unit.

For the purpose of performing the hemagglutination inhibition reaction serial dilutions of test serum are prepared (1:10, 1:20, 1:40, etc.) which has been inactivated at 58° for 20 minutes. One-tenth of a cubic centimeter of the appropriate serum dilution is put into each pocket; 0.1 cubic centimeter of the virus suspension containing four hemagglutinating units is added to it, and the fluids are mixed. Then, 0.2 cubic centimeter of a two-percent erythrocyte suspension is added to each pocket, and the fluids are mixed again and incubated in a thermostat at 37° for an hour, after which the results of the reaction are recorded. The highest serum dilution at which no hemagglutination is observed or where there are only traces of it is taken as the serum antihemagglutinin titer.

Method Recommended by the Influenza Center of the World Health Organization (Lepine, 1954). A standard suspension of erythrocytes contains 0.5 percent chicken erythrocytes or 0.5 percent human group O erythrocytes in physiological solution. The erythrocytes may be kept for a week at 4° in the form of blood to which two tenths of its volume of two-percent sodium citrate in physiological solution has been added. The suspension is prepared anew each time from erythrocytes triply eluted by centrifugation.

The standard antigen is prepared from allantoic fluid of 12-day embryos infected with viruses (the PR8, A, FM1 A-prime viruses and the Lee and Bon B virus strains are recommended). The fluid is diluted 1:4 with physiological solution, preserved, and in this form it can be kept in the refrigerator. Its titer should be no less than 1:64. For the purpose of determining the hemagglutinating unit double serial dilutions of the antigen are made in test-tubes in a volume of 0.25 cubic centimeter (the original working dilution of the antigen is prepared by means of mixing one cubic centimeter of the antigen and three cubic centimeters of physiological solution); 0.25 cubic centimeter of physiological solution and 0.5 cubic centimeter of erythrocyte suspension are added to each test-tube, and the contents of the test-tubes are mixed by shaking. The test-tubes are allowed to stand at room tempera-

ture (22-24°) for one hour. One hemagglutinating unit corresponds to the highest dilution which produces a complete hemagglutination; the volume is considered to be 0.25 cubic centimeter. For the purpose of performing the reaction a dilution of antigen is used which contains four hemagglutinating units in 0.25 cubic centimeters.

The reaction of hemagglutination inhibition is performed with sera which have been heated at 56° for 30 minutes. Double serial dilutions of serum in a volume of 0.25 cubic centimeter are mixed with 0.25 cubic centimeter of antigen containing four hemagglutinating units and with 0.5 cubic centimeter of an erythrocyte suspension. After mixing, the test-tubes are allowed to stand for one hour at 22-24°, after which the results are read.

Method Adopted at the Institute of Virology of the Academy of Medical Sciences USSR (after Gorbunova, 1953). Allantoic or amniotic fluid of chick embryos infected with various virus strains serves as the antigen. Usually, a mixture of fluids taken from several embryonic cultures of the same strain is used.

As immune serum use is made exclusively of the serum of white rats immunized intraperitoneally with undiluted allantoic fluid which is highly active with respect to its hemagglutinin content. The immunization is carried out twice at an interval of one month or three times at weekly intervals. In the former case, each rat receives the antigen in a quantity of one or two cubic centimeters; in the latter case, one cubic centimeter three times. All the rats are bled out 10-14 days after the last injection of the antigen.

Rats weighing 200-250 grams are selected; it is possible to obtain an average of about three cubic centimeters of serum from each rat. The sera from all rats immunized with a single antigen are mixed after the bleed-out, and then they are heated at 56° for 30 minutes, after which they are preserved with sodium merphenyl solution 1:50,000, and poured out into ampoules. This treatment provides for the maintenance of the neutralizing properties of the serum without reduction in titer for a very long time (no less than three years).

The main advantage of the utilization of white rats over other generally accepted laboratory animals is the absence of nonspecific inhibitors in their sera which complicate working with rabbit and ferret sera.

In working with experimental rat sera use is made of guinea-pig erythrocytes. This type of erythrocytes gives a more distinct and stable sedimentation reaction than do chick erythrocytes. The defibrinated blood of

guinea pigs taken from the heart is washed out with physiological solution by means of centrifugation of the suspension. The triply eluted erythrocyte sediment is diluted for the experiment with physiological solution in a volume relationship of 1:100.

In experiments with human sera chick erythrocytes are also used since these sera not uncommonly contain hemagglutinins to guinea-pig erythrocytes. The blood is treated by the same method as guinea-pig blood and is taken from the subalar vein of the chicken.

Before beginning the experiment a one-percent suspension of erythrocytes is prepared according to the principle that the same suspension be utilized for the establishment of the hemagglutinating titer of the antigen in the direct hemagglutination reaction as for all experiments of hemagglutination inhibition on that day.

Preliminarily titrated allantoic (or amniotic) fluids are titrated once again for the purpose of establishing the same hemagglutinating unit for this batch of diluted erythrocytes. By this method we avoid any lack of correlations in the titer with different batches of erythrocytes, which has been mentioned by Ritova and Dudkina (1950) as well as by certain foreign authors.

The reaction is performed in the ordinary laboratory test-tubes with a round bottom which are set in metal test-tube racks. Double virus dilutions aremade in a volume of 0.5 cubic centimeter, starting from the original 1:10 dilution of the allantoic fluid. In certain cases where the concentration of virus is very low, serial dilutions of the fluid are begun with 1:2. An equal volume (0.5 cubic centimeter) of a one-percent suspension of erythrocytes is added to each test-tube containing diluted virus. The mixture is carefully shaken up and allowed to stand at room temperature until a precipitate settles. The degree of the reaction is read according to a three-plus system. All the reactions which produce complete erythrocyte agglutination in which the erythrocytes are distributed as an even layer on the concave bottom of the test-tube with an undulating marginal zone which extends up the wall of the test-tube are taken as a three-plus (+++) reaction. Agglutination which causes a less uniform distribution of erythrocytes, sometimes with an even border or ring zone on the bottom of the test-tube is taken as two-plus (++). A considerable erythrocyte sedimentation as a dense disc with a granular fringe along the margin is read as a one-plus (+) reaction; sedimentation as a dense disc with smooth edges is read as a negative reaction (-).

The final virus suspension dilution which gives a distinct three-plus erythrocyte agglutination is taken as one hemagglutinating unit on condition that the next dilution produces only a one-plus (+) reaction or sedimentation of erythrocytes without agglutination (-). In the event a two-plus (++) reaction is observed after the three-plus reaction the dilution which lies between the dilutions giving three- and two-plus reactions is taken as a single hemagglutinating unit. A dilution corresponding to four hemagglutinating units is prepared for the hemagglutination inhibition experiment. For example, if the hemagglutinating unit corresponded to a dilution of 1:160, a dilution of 1:40 is taken as four units.

The serum dilutions are prepared according to the calculation that each serum participate in cross reactions with all viruses used for the given experiment. Double serial dilutions of the serum in a volume of 0.25 cubic centimeter are begun with an original dilution of 1:10 in physiological solution. If several viruses are used in the experiment the serial serum dilutions are made with a single pipette so that 1:20 dilutions are first prepared for all the viruses; then, a dilution of 1:40 is made for each virus from each test-tube having a dilution of 1:20, etc. After the dilutions of all the sera are finished, 0.25 cubic centimeter of allantoic fluid containing the virus and first diluted to a concentration corresponding to four hemagglutinating units with respect to an equal volume of a one-percent erythrocyte suspension is added to each series of serum dilutions in a volume of 0.25 cubic centimeter.

The mixture of serum with virus is carefully shaken up, and after a five-minute rest 0.5 cubic centimeter of one-percent erythrocyte suspension is added to this mixture, which also has a volume of 0.5 cubic centimeter. After consideeable shaking once again the mixture is allowed to stand at room temperature for about 40 minutes. During this period either a sedimentation of the erythrocytes occurs in the form of a circle with smooth borders, or hemagglutination occurs. Reading of the reaction is accomplished according to the same three-plus system described above.

Inhibition of hemagglutination expressed in a complete absence of a reaction or in the form of traces of agglutination not over one-plus is taken as the serum titer. A two-plus hemagglutination is considered to amount to an absence of any inhibiting effect by the serum on the capacity of the virus for producing erythrocyte agglutination.

Each virus participates in the reaction with all the sera used in the experiment; a part of them should be homologous.

Therefore, the serum titer with the homologous virus is established experimentally each time, and the reading of the heterologous titers is made according to the results of the given experiment. Through this method the differences attributable to the avidity factor (different capacities of allantoic fluid for reacting with immune serum) are partially eliminated. Nevertheless, we, like other investigators, had to admit that there was a lack of reciprocity in certain groups of crossed reactions.

The evaluation of the results is accomplished by means of calculating the ratios of the serum titers in the experiment with hetero- and homologous virus strains. In Table 13 an approximate calculation is given of the similarities and differences between the PR8, A36, Shklyaver, Pan and Kri strains with respect to the serum for the PR8 strain.

Table 13

Similarities and Differences Between the Influenza Virus Strains as Shown by the Method of Calculating the Ratio of the Heterologous Serum Titer to its Homologous Titer in the Hemagglutination Inhibition Reaction

Serum Strain	Virus	Result of Hemagglutination Inhibition Reaction Depending on Serum Dilution										Ratio of Titers
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	
PR8	A PR8	–	–	–	–	–	–	–	–	–	+++	Homologous Titer of 2560 = Unity
PR8	A A36	–	–	–	–	–	–	–	–	–	+++	$\frac{2560}{2560} = 1$
PR8	A Shklyaver	–	–	–	–	–	–	+++	+++	+++	+++	$\frac{320}{2560} = \frac{1}{8}$
PR8	A, Pan	–	–	+++	+++	+++	+++	+++	+++	+++	+++	$\frac{20}{2560} = \frac{1}{128}$
PR8	B Kri	+	+++	+++	+++	+++	+++	+++	+++	+++	+++	0

Key: +,+++ are positive hemagglutination reactions; – negative hemagglutination reaction, that is, a specific inhibition of the immune serum reaction in various dilutions.

The titer of serum against the PR8 strain corresponded to a 1:2560 dilution of it, which produced a complete hemagglutination inhibition of the homologous PR8 strain. The reaction of this PR8 strain serum with the A36 strain was identical to the preceding one, on the basis of which we can speak of the identity of both strains with respect to this serum. The ratio of the serum titer for the heterologous A36 strain to the titer for the homologous strain is equal to unity.

The hemagglutination inhibition of the Shklyaver strain with the same PR8 serum did not come up to the serum titer, ending at a dilution of 1:320. The ratio of the heterologous to the homologous titer was equal to 1/8. It may be considered arbitrarily that the similarity of antigenic components in both strains is equal to 1/8 of the antigenic PR8 unit.

The ratio of the PR8 serum titer with the Pan virus to its homologous titer is found to be 1/128, which indicates the presence of a very small amount of antigenic component in the PR8 serum equivalent to the Pan antigen. When the same ratios are calculated between the titers of the PR8 serum and the Kri virus it is impossible to establish the existence of any kind of common antigens in their structures. It should be noted that any hemagglutination inhibition in a serum dilution of 1:10 should be considered nonspecific and is not to be taken into account.

For the purpose of a more demonstrative comparison of the antigenic interrelationships between the strains it is suggested that use be made of a schematic representation of the crossed reactions (Gorbunova, 1951).

In Fig. 43 data are presented which have been transferred from appropriate tables to a grid consisting of separate squares each of which is filled in in accordance with results of crossed hemagglutination inhibition reactions. Thus, a completely filled square indicates a ratio of the heterologous titer to the homologous titer equal to unity.

On the basis of data obtained in experiments of crossed hemagglutination inhibition of A-prime and B strains by their antisera it may be considered firmly established that the hemagglutinin of the influenza virus is a carrier of the type specificity of the virus and that by means of rat immune sera it is easy to determine the serological type of the influenza virus as well as differences between the strains within the type.

In a number of works there have been attempts to give accurate quantitative methods for determining the influenza hemagglutinin (Levine, Puck and Sagik, 1953; Bely-

avin and others, 1951).

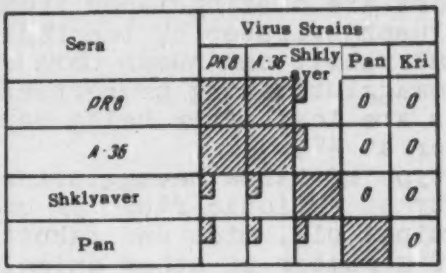

Fig. 43. Diagram of Antigenic Interrelationships Between Strains According to Their Antisera. The Completely Shaded Squares Correspond to Homologous Titer of Each Serum and to Heterologous Titers When the Strains are Identical.

The squares which are hatched to the extent of 1/8 of their area indicate that the titer of the given serum with heterologous virus strain amounts to 1/8 of its homologous titer; the zero indicates the figures which constitute fractions less than 1/64 (Gorbunova, 1953).

The squares which are hatched to the extent of 1/8 of their area indicate ratios which, as a result of division of the heterologous serum titers by the homologous serum titers, gave a fraction equal to 1/8.

In the square which represents the reaction between the Pan virus and the PR8 serum a ratio of the titer in this reaction of less than 1:64 is designated by a zero. The zero designates also a lack of a hemagglutination inhibition reaction between the Kri virus and all the sera mentioned.

In performing the hemagglutination reaction with influenza viruses one sometimes runs up against false-positive reactions which depend on the fact that various substances and living agents may possess hemagglutinating properties. This problem will be analyzed in greater detail in the chapter on the diagnosis of influenza; here we shall present certain data pertaining to the false hemagglutination reaction which is encountered in the isolation of influenza viruses from chick embryos.

These reactions are often observed with bacterial contamination of the chick embryos during the course of isolating the influenza viruses from nasopharyngeal washings. Florman (1946) points out that the titer of these

reactions may reach 1:160. The cause of these reactions is the presence of gram-positive or gram-negative cocci as well as B. subtilis in the allantoic fluid. These false-positive reactions are distinguished from hemagglutination produced by influenza viruses by the fact that they are inhibited by normal rabbit and human sera and also by the fact that the hemagglutinating properties of contaminated allantoic fluids are lost after being kept for four days in a refrigerator at 4°.

Another type of false hemagglutination is connected with the property of amniotic fluid of chick embryos of agglutinating buinea pig, mice and rabbit erythrocytes, as well as those of a number of other animals but not of chickens. Agglutination is observed usually in low titers and is produced by the globulin protein fraction. The hemagglutinin is not present in allantoic fluid. It is thermolabile, being destroyed even at 48° in 20 hours. The hemagglutinin is not neutralized with specific sera. Therefore, the false hemagglutination produced by the amniotic fluid is comparatively easily distinguished from the hemagglutinin produced by influenza viruses (Commission on Acute Respiratory Diseases, 1946).

20. Characteristics of the Hemagglutination Reaction. Reaction Phases

The agglutination of erythrocytes by influenza viruses is a complex reaction consisting of three phases: in the first phase adsorption of the virus by the erythrocytes occurs; in the second, the agglutination of aggregates of the virus and of the erythrocytes; in the third, the elution of the virus from the erythrocytes (Hirst, 1942). The adsorption of the virus onto erythrocytes occurs rapidly. The rapidity does not depend on the temperature, the virus concentration or the concentration of the erythrocytes. Under ordinary conditions of performing the reaction the process of virus adsorption onto erythrocytes is accomplished in a few minuts, after which agglutination of the erythrocytes occurs. The duration of this stage ranges from several minutes to several hours. Elution of the virus, which is accomplished over the course of several hours (Fig. 44), occurs most slowly.

After being in contact with the influenza virus the erythrocytes lose their capacity of adsorbing it again. Therefore, in a mixture of such erythrocytes with the influenza virus neither adsorption nor agglutination occurs (Fig. 45). These data, which have been established by Hirst (1942) have, in general, been confirmed by subsequent investigations.

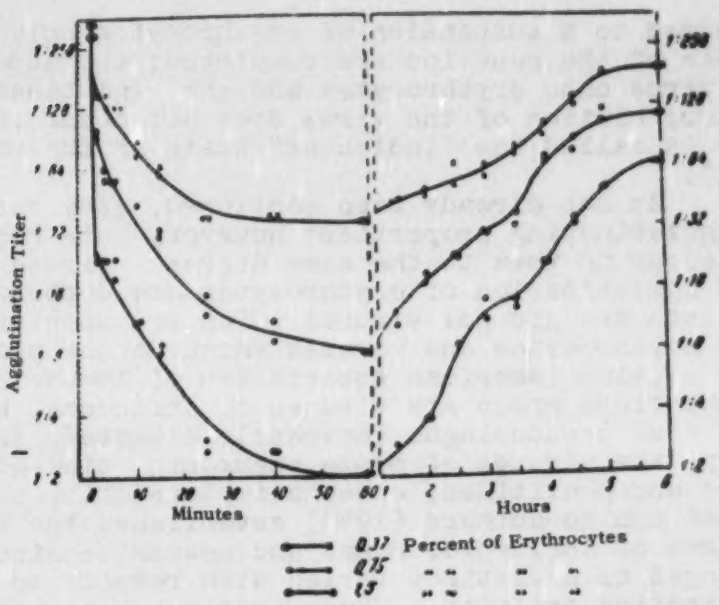

Fig. 44. Agglutination Titer in Fluid Containing Influenza Virus A (PR8) After the Addition of Erythrocytes. The reaction was performed at 23° (Hirst, 1942).

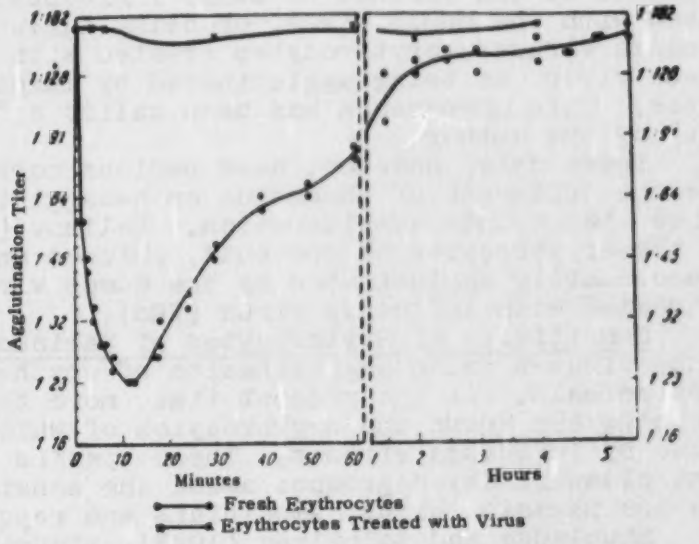

Fig. 45. Agglutination Titer in Fluid Containing Influenza B Virus After the Addition of Fresh and Virus-Treated Erythrocytes. The reaction was performed at 24° with 0.75 percent erythrocytes (Hirst, 1942).

If the influenza virus which has been heated at 56°

is added to a suspension of erythrocytes only the two first phases of the reaction are completed: the adsorption of the virus onto erythrocytes and the agglutination of the latter; elution of the virus does not occur (Briody, 1948). This is called the "indicator" state of the virus (Burnet, 1951).

As has already been mentioned, many viruses possess hemagglutinating properties; however, this process does not occur in them to the same degree. Viruses which produce agglutination of erythrocytes are customarily divided into two groups: viruses which are spontaneously eluted from erythrocytes and viruses which do not produce spontaneous elution (American Association of Immunologists, 1951). In the first group are viruses of influenza, mumps and Asiatic fowl pseudoplague [Newcastle disease]; in the second group, the viruses of mouse pneumonia, tick-borne and mosquito encephalitides, encephalomyocarditis, and GD VII. Burnet and co-authors (1946) established the fact that the viruses of influenza, mumps and newcastle disease can be arranged as a distinct series with respect to their hemagglutinating activity. Human erythrocytes treated with the virus of swine influenza lose their capacity of being agglutinated by all the viruses named; erythrocytes treated with the influenza A virus lose the capacity of being agglutinated by the viruses of swine influenza; erythrocytes treated with the mumps virus, of being agglutinated by the influenza viruses; erythrocytes treated with the newcastle disease virus, of being agglutinated by mumps and influenza viruses. This phenomenon has been called a "receptor gradient" by the author.

These data, however, need serious corrections, because the influence of themedium on hemagglutination has not been taken into consideration. Selimov (1954) showed that the erythrocytes of the bull, chicken and young pig are secondarily agglutinated by the mumps virus after being treated with influenza virus (PR8).

Sensitivity of Erythrocytes of Various Animals. Influenza viruses cause agglutination of erythrocytes of different animals. At the present time, more than 20 species of animals are known the erythrocytes of which are agglutinated by influenza viruses. These species belong to different classification groups: among the sensitive animals there are mammals, birds, amphibians and reptiles.

Shubladze and Solov'yev (1943), studying the sensitivity of erythrocytes of various animals to agglutination by influenza virus, showed that erythrocytes of chickens, guinea pigs, dogs and man are very sensitive (++++); erythrocytes of white rats and white mice are not very sensitive

(++); erythrocytes of the horse, sheep, fox, cat, rabbit and frog are not agglutinated. The blood group to which the human erythrocytes belong has no effect on their sensitivity to the influenza virus. According to the data of Twbyle and Mason (1944), the erythrocytes of chickens, ducks, pigeons, turkeys, guinea pigs and man are agglutinated by the influenza virus, while the erythrocytes of rabbits, rats, mice and sheep are not agglutinated.

Erythrocytes of chickens, guinea pigs and man (Clark, Nagler, 1943) possess the greatest sensitivity to hemagglutination. Selimov (1954) compared the hemagglutinating properties of influenza viruses (PR8 strain) and of mumps and found that the former causes the agglutination of 14 out of 29 tested species and the latter, of 22 out of 29. We are presenting a table taken from the work of Selimov (Table 14).

Table 14

Sensitivity of Hemagglutination Reaction of Erythrocytes of Various Animals in Influenza A and Mumps Viruses

No.	Erythrocytes	Mumps virus	Influenza Virus A	No.	Erythrocytes	Mumps Virus	Influenza Virus A
1	Chickens	+	+	16	Guinea pigs	+	+
2	Geese	+	+	17	Dogs	+	+
3	Domestic Duck	+	+	18	Raccoons	+	—
4	Wild Duck	+	+	19	Foxes	—	—
5	Caucasian pheasant	+	+	20	Young pigs	+	—
6	Eagles	+	+	21	Asses	—	—
7	Sea gulls	+	+	22	Cats	+	—
8	Owls	+	+	23	Horses	+	—
9	Buzzards	+	+	24	Ponies	+	—
10	Peacocks	—	—	25	Sheep	—	—
11	Lake frogs	—	—	26	Squirrels	—	+
12	Amphibian frogs	+	+	27	White mice	±	±
13	White rats	±	±	28	Man	+	+
14	. .	±	±	29	Monkeys	+	—
15	Rabbits	+	—				

Key: + intense reaction; ± weak reaction; - no agglutination.

Similar data have been obtained by Chu (1948) who made similar investigations. The PR8 and Lee influenza viruses agglutinated the erythrocytes of birds (chickens, ducks, daws), man, guinea pig, ferret, rat, dog and hedgehog better than did the virus of swine influenza. Heated PR8 and Lee viruses (at 36° for 30 minutes) agglutinated the erythrocytes of the sheep, bulls and goats better than did the natural viruses. Makower and Skurska (1951), in a study of the agglutinability of different species of erythrocytes by influenza viruses, showed that sheep erythrocytes behave differently; some specimens of them are not agglutinated; others are agglutinated only at 4°; still others, at 4° and 20°; cow erythrocytes are agglutinated only at 4° and in low titers; the erythrocytes of mice, rats and rabbits are not agglutinated.

The Effect of the Environmental Conditions on Agglutination. According to the data of Miller and Stanley (1944), in the performance of the reaction variations in the concentration of erythrocytes within the limits of from 0.43 to 3 percent do not exert any essential influence on the results of the reactions; the age of chickens from which the erythrocytes are taken is not of essential importance, but erythrocytes taken from various chickens produce variations in the reaction titer up to 36-38 percent under otherwise equal conditions. Dreyzin (1952) points out that the intensity and rapidity of virus adsorption increase markedly with the increase in the concentration of the erythrocytes. The increase in the erythrocyte concentration leads to a decrease in the rate and degree of elution of the virus.

Salk (1944), using various erythrocyte concentrations (from 0.375-0.06 percent) in performing the test with the same virus concentration observed variations in the reaction titers from 640 to 5120. When the concentration of erythrocytes dropped to 0.03 percent the reaction became indistinct and could not be recorded. The influence of quantitative relationships of the components was studied again by Romanenko and Khayt (1953), from whose works we are presenting the following data (Table 15).

Therefore, a suspension of 0.5-1 percent erythrocytes (calculating for the original blood volume) is the optimum. Thick suspensions are poorly suited to the detection of small quantities of virus; dilute suspensions give poorly demonstrable results. In special experiments the authors showed that the reaction depends not so much on the virus concentration as on the total quantity of it in the volume being examined. Therefore, up to certain limits the components may be diluted without changing the results of the

reaction (Table 16).

Table 15

Results of Titration of the Same Influenza Virus Suspension in Parallel Experiments with the Use of Erythrocyte Suspensions of Different Concentrations

Concentration of Erythrocyte Suspension in %	Dilution of Virus Suspension							
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
0,2	+	±	±	±	±	±	–	–
0,5	+++	+++	+++	+++	++	+	±	–
1,0	+++	+++	+++	+++	++	±	–	–
2,0	+++	+++	+++	++	+	–	–	–
4,0	+++	+++	++	+	+	–	–	–
8,0	+++	+++	+	–	–	–	–	–
16,0	+++	±	–	–	–	–	–	–
32,0	–	–	–	–	–	–	–	–

Table 16

Results of Experiments For the Detection of a Single Agglutinating Dose of the Virus Depending on the Degree of Dilution of the Original Suspension of it With Physiological Solution

Material being examined	Presence of Hemagglutination
A single agglutinating dose of the virus in a volume of 0.5 cubic centimeter + 0.5 cubic centimeter of a suspension of one percent erythrocytes.	+++
The same + 0.5 cubic centimeter of physiological solution.	+++
. . +1 . . .	+++
. . +2 . . .	+++
. . +4 . . .	++
. . +8 . . .	++
. . +16 . . .	±
. . +32 . . .	–

The intensity of the hemagglutination reaction depends on the temperature. According to Salk's data (1944), the hemagglutination titers are increased at low temperatures (2°) over what they are at the higher temperatures (28°); at a temperature of 35° the reaction is so indis-

tinct that it cannot be recorded. Miller and Stanley (1944), performing experiments with the same components, obtained different hemagglutinating virus titers (PR8 strain) at different temperatures: if the hemagglutination titer at 24° is taken as 100 the levels of it at 4° and 35° respectively were equal to 170 and 33. In making the calculations the author obtained an empirical formula for the relationship of the hemagglutination titer to the temperature:

$$T_{25^\circ} = \frac{100}{100 + (t - 25) \cdot 4.5} Tt,$$

where T_{25° is the reaction titer of 25°; Tt is the reaction titer at a given temperature.

According to the data of Makower and Skurska (1951), in performing the hemagglutination reaction with various influenza virus strains different results areobtained: for the PR8 strain the hemagglutination reaction titer is the same at 20° and at 4°, and for the PR8 strain at 4° it is higher than at 20°.

According to the data of Dreyzin (1952), a low temperature is optimal for adsorption of the virus if the results are considered with respect to the highest dose of virus adsorbed. At room temperature and particularly at a thermostat temperature the adsorption is accomplished more rapidly, but the absolute quantities of adsorbed virus are less than at 0-4°. On the strength of this fact the erythrocytes of sheep, rabbits, mice and rats are well agglutinated by the influenza virus if the experiment is performed at 0-2°. The intensity of elution is increased markedly with the increase in the temperature. It is negligible at a temperature close to 0°, moderate at room temperature, and is intense at 37-50°. What has been stated is well illustrated by the adsorption and elution curves of the influenza virus at various temperatures (Fig. 46) obtained by Hirst (1942).

The reaction of the medium does not have any essential influence on the degree of hemagglutination within the limits of a pH from 6-9 (Miller and Stanley, 1944; Peterson, 1949). These pH variations do not reflect much on the quantity of virus taken up by the erythrocytes in a medium with an optimum electrolyte concentration. In an acid (pH lower than 5) and an alkaline (pH over 10) medium a rapid inactivation of the hemagglutinating properties of the influenza virus occurs (Yeremeyev, 1952). Here, the hemagglutinating activity is lost more rapidly in an acid medium than in an alkaline medium (Miller, 1944).

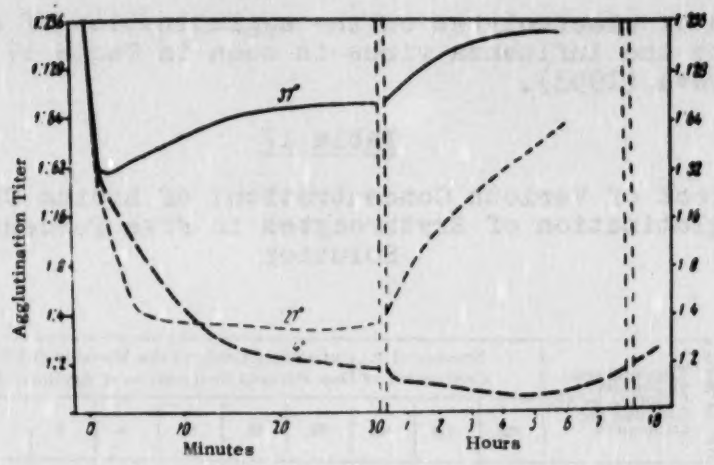

Fig. 46. Titers of Fluid Containing Influenza A (PR8) Virus at Various Temperatures (Hirst, 1942).

According to Yeremeyev's data (1952), the binding of the virus by the erythrocytes and the strength of the bond are determined by the electrolyte composition of the medium. The quantity of virus taken up by a definite dose of erythrocytes is minimal in the absence of salts and maximal in a 0.5 percent concentration of sodium chloride or of 0.02 percent calcium chloride. An excess of salts (concentrated solutions) reduces the intensity of the influenza virus reaction with the erythrocytes and their adsorptive capacity. Uni- and bivalent cations, by changing the surface of the virus and erythrocytes, increase the reversible reaction of them. Trivalent cations in an insignificant concentration contribute to an irreversible bond of the virus with the erythrocytes, and with a higher concentration of them the virus is completely inactivated. When electrolytes are present in the medium an excess of non-electrolytes does not exert any influence on the uptake of virus by the erythrocytes.

The decrease in the sodium chloride concentration by dialysis of a suspension of erythrocytes saturated with the virus leads to a liberation of the B virus (but not of the A virus) and of the virus of swine influenza. When the concentration of sodium chloride is increased to five percent all the species of viruses are eluted in an intensified manner.

Strong (5-10 percent) solutions of sodium chloride have no effect on the binding of the virus by erythrocytes but prevent hemagglutination. The influence of the concen-

tration of electrolytes on the agglutination of erythrocytes by the influenza virus is seen in Table 17 taken from Yeremeyeva (1953).

Table 17

The Effect of Various Concentrations of Sodium Chloride on the Agglutination of Erythrocytes in Five Percent Glucose Solution

No. of Series	Concentration of Sodium Chloride in 5% Glucose Solution in %	Number of Agglutinating Units of the Virus in 0.5 Cubic Centimeter of One Percent Suspension of Erythrocytes										
		400	200	100	50	25	16	8	4	2	1	0
1	0	4	0	0	0	0	0	0	0	0	0	0
2	0,001	4	0	0	0	0	0	0	0	0	0	0
3	0,01	4	4	4	0	0	0	0	0	0	0	0
4	0,05	4	4	4	4	4	4	0	0	0	0	0
5	0,1	4	4	4	4	4	4	4	4	0	0	0
6	0,4	4	4	4	4	4	4	4	4	4	4	0
7	0,85	4	4	4	4	4	4	4	4	4	4	0
8	2,5	4	4	4	4	4	4	0	0	0	0	0
9	5	4	4	4	4	4	0	0	0	0	0	0
10	Control (physiological solution)	4	4	4	4	4	4	4	4	4	4	0

Key: 4 -- erythrocyte agglutination; 0 -- no agglutination.

The lower limits of ion concentration at which hemagglutination is possible are equal to 0.014 molar for sodium chloride and 0.0067 molar for magnesium chloride; the bivalent salts permit lower concentrations than do the univalent salts (Burnet and Edney, 1952). Complex colloidal ions in low concentrations inhibit the hemagglutination of the erythrocytes by the virus, and in high concentrations they themselves can agglutinate the erythrocytes (Miyamoto, Akama, Yoshizu, 1950).

The optimum medium for eaction of the virus and erythrocytes is a 0.1 molar phosphate buffer containing an isotonic solution of dextrose or serum albumin, although the reaction may proceed also in 0.03-0.17 molar solutions of sodium chloride (Ryan; Evans and Melnik, 1951).

For the purpose of carrying out the hemagglutination

reaction the presence of calcium ions, with which the enzymic activity of influenza viruses is associated is necessary; more will be said about the latter below (Portfield, 1952; Nihoul, 1952).

Electron microscopic observations of the process of adsorption onto an elution of the virus from erythrocytes give the following pictures. The virus particles are adsorbed onto the surface of erythrocytes not in a solid, continuous form, but rather at various places (Figs. 47 and 48). After the elution of the virus particles from the surface of the erythrocyte depressions and hollows remain as the result of which the surface becomes rough (Fig. 49). Along with the solitary particles or depressions corresponding to them accumulations of virus particles or large, irregular hollows corresponding to them are encountered (Ryan, Evans and Melnik, 1951).

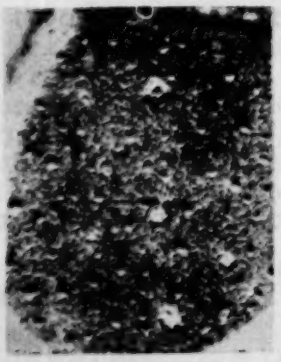

Fig. 47. Adsorption of Influenza Virus onto Erythrocyte After 15 Minutes at 25° (Ryan and others, 1951).

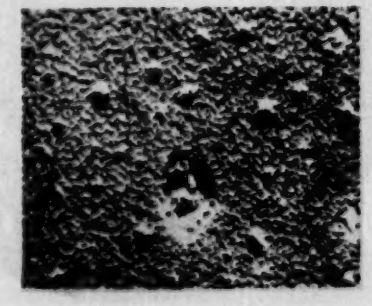

Fig. 48. Section of Erythrocyte Depicted in Fig. 47 with Higher Magnification (Ryan and others, 1951).

Fig. 49. Hollows on the Surface of an Erythrocyte After Elution of the Virus (Ryan and others, 1951).

With the use of the erythrocyte stroma as an adsorbent it is possible to obtain particularly demonstrative pictures of influenza virus adsorption onto erythrocytes. In Figs. 50 and 51 electron microscopic pictures of adsorption of A-prime and B viruses onto chick erythrocytes are shown taken from the collection "Influenza" (Geneva, 1954). The presence of filamentous and rod-like forms in the preparations of the A-prime viruses attracts attention.

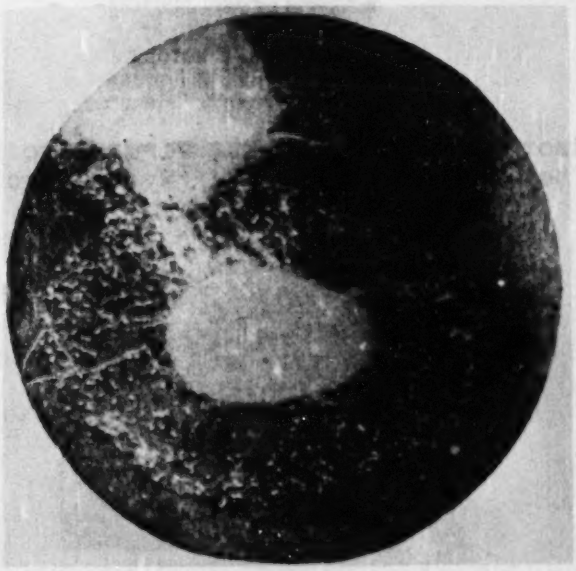

Fig. 50. Virus of A-Prime Influenza Adsorbed onto Stroma of Chick Erythrocyte. The Elementary Bodies and Filaments (Influenza Center, 1954).

Fig. 51. Virus of B Influenza Adsorbed onto Stroma of Chick Erythrocyte. Elementary Bodies (Influenza Center, 1954).

Depending on the characteristics of the virus preparation and the methods of treatment of them the electron microscopic pictures may show a considerable variety of virus forms. In Figs. 52-55 electron micrographs of the adsorption of influenza virus onto erythrocytes obtained by Draganov (1954) at the Institute of Virology of the Academy of Medical Sciences USSR are presented. In Fig. 52 there are only spheroidal particles of the influenza virus scattered more or less uniformly along the surface of the erythrocyte. In Fig. 53 spheroidal, filamentous and racket-shaped (NV bodies) forms of the virus are presented. The latter are predominant in Fig. 54. In Fig. 55 elution of the virus has partially occurred, and injuries to the surface of the erythrocytes remain in the form of crater-like depressions. These electron micrographs give a graphic representation of the morphologic expressions of the reaction of the influenza virus with erythrocytes during the course of adsorption and elution.

The data presented as well as the investigations of Pokotinskiy and Luzyanina (1953) indicate that the influenza virus is not always uniformly distributed along the surface of the erythrocyte, and, depending on the adsorption conditions, can be adsorbed in the form of aggregates of elementary bodies, filaments or other structures. This

needs to be very critically applied to the statements by Sagik (1954) to the effect that the erythrocyte has a limited number of points on its surface to which the virus of influenza may be attached.

Fig. 52. Section of Chick Erythrocyte with Adsorbed B Virus (Kri). Shadowing with Chromium. Magnification 11,000 (Draganov, 1953).

Fig. 53. Chick Erythrocyte with Adsorbed A-Prime Virus (Hall). Elementary Bodies and NV Bodies. Shadowing with Chromium. Magnification 13,000 (Draganov, 1953).

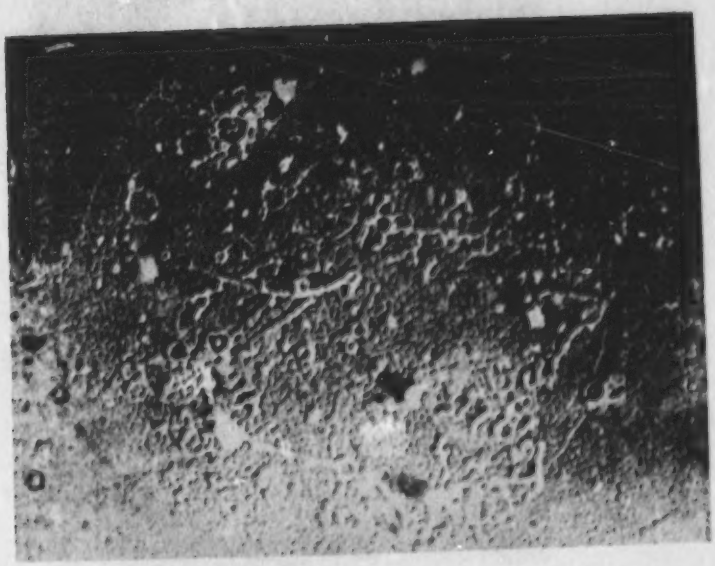

Fig. 54. Virus of A-Prime Influenza (Hall) Adsorbed onto Stroma of Hemolyzed Erythrocyte. Elementary Bodies and NV Bodies. Magnification 14,000 (Draganov, 1954).

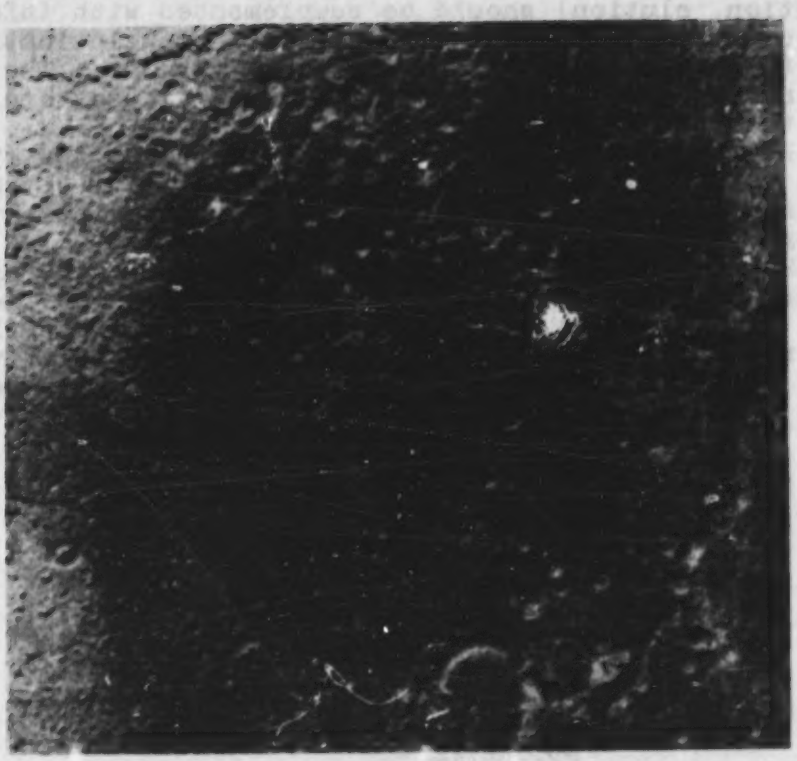

Fig. 55. Erythrocyte After Partial Elution of the Virus (The Depressions Are Seen) (Draganov, 1954).

21. Properties of the Hemagglutinin

Resistance. The data presented above concerning the influence of the conditions of the medium on the hemagglutination reaction and its various phases (adsorption, agglutination, elution) should be supplemented with information concerning the resistance of the hemagglutinating factor (hemagglutinin) of the influenza viruses. The hemagglutinating properties of the influenza viruses are more stable than the infectious properties.

Heating of the material containing virus for 15-30 minutes at 56°, at which the infectious activity of the virus is destroyed, does not destroy its hemagglutinating activity (Peterson, 1949).

Zakstel'skaya (1953) compared the resistance of infectious, toxic and hemagglutinating properties of the influenza A virus and showed that the first two properties are thermolabile, whereas the hemagglutinin is relatively stable, withstanding heating to 60° and being destroyed on boiling (Fig. 56).

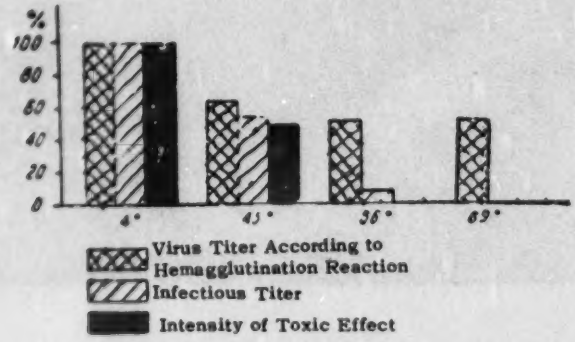

Fig. 56. Change in the Properties of the Influenza Virus on Heating (Zakstel'skaya, 1953).

Similar data have been obtained in the study of the effect of sunlight and ultra-violet irradiation. Direct sunlight almost completely destroys the toxic and infectious properties after 18-24 hours, whereas the hemagglutinin titer decreases only moderately after such an effect (Fig. 57). Ultra-violet irradiation of the virus in a cubicle with a lamp of monochromatic type (wave length 2350 Å ; the distance from the lamp to the flask containing the virus is 30 centimeters) destroys the infectious activity and the toxic properties of the influenza virus in 15 minutes, whereas the hemagglutinating activity of it is not much impaired (Fig. 58).

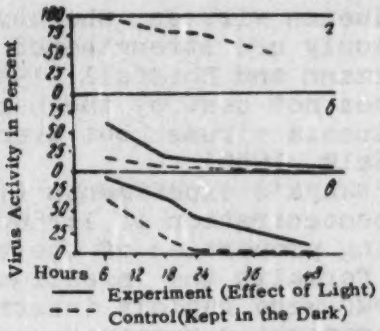

Fig. 57. Effect of Sunlight on Influenza Virus Properties a -- change in hemagglutinating properties; b -- change in infectious properties; c -- change in the toxic properties (Zakstel'skaya, 1953).

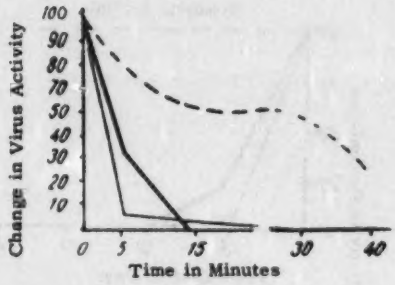

Fig. 58. Change in the Influenza Virus Properites Under the Effect of Ultra-Violet Rays.

Key: --- hemagglutinating properties. toxic properties infectious properties (Zakstel'skaya, 1953).

Ultra-violet irradiation (the source of irradiation is a Bach lamp; the distance from the source to the material being irradiated -- a suspension of mouse lungs -- is 25 centimeters) does not reduce the hemagglutinating activity of the virus following a $1\frac{1}{2}$ hour exposure time, although in this time the infectious activity of the virus disappears (Peterson, 1949).

More precise investigations show that the infectious activity is destroyed chiefly by the portion of the spectrum having a wave length of about 2652 Å; the agglutinating activity, by 2803 Å (Tamm and Fluke, 1950).

Through the cautious effect of ultra-violet rays and lanthanum it is possible to interfere with the phase

of elution in influenza viruses, whereby the properties of being eluted slowly are strengthened in subsequent virus generations (Björkmann and Horsfall, 1948).

Formalin does not destroy the hemagglutinating activity of the influenza viruses but even increases their thermostability (Salk, 1946).

In Zakstel'skaya's experiments (1953) the addition of formalin in a concentration of 1:2500-1:5000 stabilized the hemagglutinating properties of the virus. With these concentrations of formalin the infectious and toxic properties of the virus were rapidly inactivated, whereas the hemagglutinating properties remained unchanged after 20 days (Fig. 59). When the virus suspension is kept for a long time in the refrigerator (4-6°) the hemagglutinating activity of the fluid remains almost constant up to 90 days (reduction of the titer by 50 percent), whereas the infectious activity falls almost to zero after 10 days.

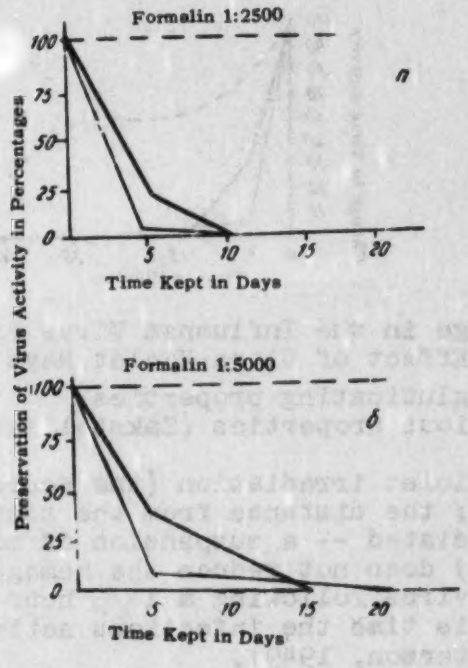

Fig. 59. Change in Influenza Virus Properties Under the Influence of Formalin.

Key: Same as Fig. 58. (Zakstel'skaya, 1953).

All these observations indicate that the hemagglu-

tinating properties are not connected with the presence of live virus; they can be preserved in the inactivated influenza virus.

Hemagglutinating and infectious properties in experimental infection produced by influenza viruses usually coincide, which is expressed in the existence of a proportionality between the corresponding virus titers (Hirst, 1942). Thus, in comparing the infectious and hemagglutinating activity of virus contained in mouse-lung suspensions infected with the PR8 strain, their infectious titer was equal to $10^{-6.3}$ - $10^{-6.8}$ LD_{50}; the hemagglutinating titer, 1:960-1:1280; the respective titers of allantoic fluid were $10^{-6.0}$ and 1:320; the titers of chick embryo tissues were, respectively, $10^{-4.9}$ - $10^{-5.2}$ and 1:15-1:20. The logarithm of the ratios of the values of the infectious and hemagglutinating titers ranged within limits of 3.5-3.9, that is, it is practically of a constant magnitude. Similar data were obtained through the comparison of four influenza virus strains, although the variations here were greater (Hirst, 1941).

After the infection of mice with pathogenic influenza virus strains a parallelism is observed between the hemagglutination titer, the infectious titer and the pulmonary pathology (Twyble and Mason, 1944).

In allantoic cultures in chick embryos a parallelism is observed between the increase in the infectious and hemagglutination titers: the increase occurs quickly during the first few days of incubation; after four days a critical drop occurs in the titers, and the hemagglutinins completely disappear on the seventh day (Gorbunova, 1949).

It was shown through special investigations that one agglutinating dose corresponds to $10^{6.2}$ LD_{50} of the virus in allantoic fluid (Cairns, Fazekas De St. Groth; and Edney, 1952).

Afterwards, however, observations of the opposite nature began to accumulate indicating the absence of a parallelism between the hemagglutinating and infectious activity of the influenza viruses.

Facts have been mentioned above which indicate different resistances of the hemagglutinating and infectious factors. Another group of facts has been obtained in experiments with the adaptation of influenza viruses to the organisms of experimental animals. In passages of the influenza virus in ferrets the virus accumulates in the discharge from the respiratory tracts in considerable concentrations, whereas its hemagglutinating activity is equal to zero. Tovarnitskiy (1951) notes that this fact, which is of importance in principle, has remained unnoticed by

the majority of investigators. Such observations have been noted repeatedly in experimental influenzal infection in mice. They are well known to every virologist working with influenza viruses in mice. In the experiments of Zakstel'-skaya (1951), who carried out investigations in our laboratory, it was established that after infection of mice with allantoic cultures of influenza viruses the latter accumulate in the lungs up to titers of 10^{-7} - 10^{-9}, without producing any pathology in the lungs and without showing any hemagglutinating activity. The latter occurs only with serial passages of the virus in mice, when thevirus becomes virulent during the course of adaptation to the mouse organism and causes pulmonary pathology. Therefore, the data noted concerning the parallelism between the infectious and hemagglutinating activity of the influenza viruses in experiments on mice are justified only for strains which are virulent for mice; for the avirulent strains the opposite rules and regulations apply: high infectious titers with zero hemagglutinating activity. Very similar data have been obtained by Solov'yev and Marennikova (1949). By comparing the strains adapted to chick embryos and to mice in experiments on mice they showed that both varieties of strains multiply in the same titers in mouse lungs, which is shown by direct titration of the virus in chick embryos and by the performance of the complement-fixation reaction. At the same time, the former do not cause any pulmonary pathology and do not produce the hemagglutination reaction, whereas these properties are manifested in the latter.

The next group of facts pertains to experiments with the purification of influenza viruses by various methods, as the result of which preparations have been obtained with the same infectious and different hemagglutinating activity. Thus, Sharp and others (1944) purified influenza virus preparations by the methods of ultracentrifugation and erythrocyte adsorption. Both methods made it possible to obtain a preparation with approximately the same infectious activity (in experiments of titration of the virus against chick embryos), but preparations obtained according to the second method had only half the hemagglutinating activity. Even more clear-cut results in this direction were obtained by Knight (1946). The author obtained purified preparations of influenza virus from mouse lungs taken from freshly killed mice after being kept at low temperatures (-70°). The infectious titers of both preparations were the same ($10^{-11.7}$ and $10^{-11.8}$); the hemagglutinating titers were very different: in the first preparation, calculating per milligram of nitrogen, the titer was equal to 5040; in the

second preparation, to 29,800 hemagglutinating units. Although certain authors (Gottschalk and Perry, 1951) were unable to separate the hemagglutinin from the virus particle, Briody (1948) succeeded in obtaining positive results in experiments with the filtration of allantoic fluid containing thevirus through Chamberland L_5 filters. All the hemagglutinins were retained on the filter. Filtrates did not have any hemagglutinating properties, although they maintained their infectious activity. Moreover, infection of chick embryos with filtrates led to a greater accumulation of hemagglutinins than did infection with the natural allantoic fluid (Table 18).

Table 18

Infectious and Hemagglutinating Activity of Allantoic Influenza Virus Cultures, Unfiltered and Filtered Through Chamberland L_5 Candles

No. of Experiments	Titer	Original Allantoic Fluid	Filtrate
1	Hemagglutination (original)	1:340	0
	Hemagglutination of allantoic fluid of infected embryo	1:340	1:1920
2	Hemagglutination (original)	1:256	0
	Hemagglutination of allantoic fluid of infected embryo	1:96–1:320	1:1920–1:2500

Sokolov and Lozhkina (1949), using the method of virus adsorption onto rabbit erythrocytes (which are not agglutinated by the influenza virus), separated the infectious from the hemagglutinating activity, on the basis of which they believe that hemagglutinins are a metabolic product of cells affected by the virus or of the virus itself.

Finally, a fourth group of facts applies to the energy relationships between the infectious and the hemagglutinating activity of influenza viruses. Lauffer and others (1946) showed that with the destruction of the infectious and agglutinating activity the energy of activation and the entropy are different (Table 19)

According to the data of these authors, the destruction of the infectious activity proceeds according to a first-order reaction, and does not so proceed for the hemagglutinating activity.

Table 19

Energy of Activation and Entropy After Destruction of Infectious and Hemagglutinating Activity of Influenza Virus

Activity	Activation Energy Cal/Mole	Entropy Cal/Mole Degrees
Infectious	34 000	39
Hemagglutinating	110 000	300

In evaluating these data, Tovarnitskiy (1951) draws the conclusion that the infectious and hemagglutinating activity of the influenza virus are produced by reactions absolutely independent of one another in which different centers of the virus particle are involved. He points out the need for recognizing a separate existence of the phenomena of biological infectious and hemagglutinating activity and notes that the greater the biological activity of the virus the stronger the course of the reaction in which the cell proteins are changed; therefore, a correlative relationship is established between the biological activity and the hemagglutination reaction. However, the virus as a biological entity possessing a definite virulence should not be confused with a secondarily acquired characteristic.

We shall return to the discussion of this problem by analyzing the theories of the nature of the hemagglutinin; here it is important to emphasize the indisputable facts indicating the absence of an identity between the infectious and hemagglutinating activity of influenza viruses and the possibility of separating these properties.

The immunologic specificity of the hemagglutination reaction was established immediately after the discovery of the phenomenon. Hirst (1941) showed that immune influenza antisera mixed with the corresponding virus strains inhibit hemagglutination when used in high dilutions. The hemagglutination inhibition occurs through the reaction of the immune serum with the virus strain corresponding to the serologic type of virus to which the immune serum has been obtained. Cross hemagglutination-inhibition reactions occur only with similar serological types of virus. A direct relationship exists between the neutralization titers of the sera when they are titrated against mice and

in the hemagglutination-inhibition reaction.

As an illustration we are presenting the results of one of the experiments taken from the quoted work of Hirst (Table 20).

Table 20

Relationship of Serum Titers in Neutralization Experiments on Mice and the Hemagglutination Inhibition Reaction After Hirst (1941)

Serum	Virus	Serum Titer in Hemagglutination Inhibition Reaction	Serum Titer in LD_{50} for Mice
PR8	PR8	1:6 000	1:16 000
	WS	1:1 024	1:1 024
	Swine	1:96	1:32
	Lee	1:32	" "
WS	PR8	1:768	1:256
	WS	1:3 000	1:2 048
	Swine	1:256	1:12
	Lee	1:32	1:4
Swine	PR8	1:64	1:4
	WS	1:768	1:16
	Swine	1:4 000	1:1 024
	Lee	1:32	1:4
Lee	PR8	1:32	1:4
	WS	1:32	1:4
	Swine	1:32	1:4
	Lee	1:4 000	1:8 000

McClelland and Hare (1941) established the existence of the property of convalescent sera of neutralizing the hemagglutinating activity of viruses of the serologic types by which the diseases were produced. In the investigation of paired sera taken at the beginning of the disease and during the period of convalescence an increase in antihemagglutinin titer was noted, which corresponded to a rise in the titers of the neutralizing antibodies found in experiments on mice. A parallel increase in the antihemagglutinins and virus-neutralizing antibodies to the corresponding type of virus was noted also in persons vaccinated against influenza.

Similar data were obtained by Shubladze and Solov'yev (1943). In Table 21 data are presented which indicate the high degree of correlation between the infectious and

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the hemagglutination-inhibiting activity of influenza antisera.

Table 21

Immunological Specificity of Hemagglutination Reaction (According to Shubladza and Solov'yev, 1943)

Dilution		Hemagglutination Reaction		Neutralization Experiment	
Virus	Serum	With Influenza Antiserum	With Normal Serum	Mice Infected With Mixture of Virus and Immune Serum	Mice Infected With Mixture of Virus and Normal Serum
1:100	1:2—1:32	—	++++	3/0	3/3
	1: 64	++	++++	3/0	3/3
	1:128	++++	++++	3/0	3/3
	1:256	++++	++++	3/2	3/3
1:100	Whole	—	++++	3/2	3/3
1:1000	»	—	+++	3/2	3/3
1:10 000	»	—	—	3/2	3/3
1:100 000	»	—	—	3/0	3/0

Key: The numerator represents the number of mice in the experiment; the denominator, the number of mice which died.

The data presented have been repeatedly confirmed by subsequent investigations, and the method of hemagglutinin neutralization has become widespread in the immunology of influenza and in the study of the antigenic structure of influenza viruses because of its simplicity and economy.

It should be noted, however, that just as the hemagglutinin is not identical with the virus particle which possesses infectious activity the antibodies which neutralize the hemagglutinin (antihemagglutinins) are not identical with the antibodies which neutralize the infectious activity of the influenza viruses. The negative correlation between the antihemagglutinin titers and the virus-neutralizing antibodies has been noted repeatedly (Francis, 1947; Walker and Horsfall, 1950; Gorbunova, 1953). Afterwards, it was shown that this lack of correlation has various causes: on the presence of hemagglutination inhibitors in the sera, on the properties of the virus preparations used, on the nature and times of mixing the ingredients (Romanenko and Khayt, 1953) and on a number of other factors which will be analyzed in detail in this and subsequent chapters. At the same time, these data have not

reduced the significance of the hemagglutination reaction as the most important method of studying the biological properties of the influenza virus, the pathogenesis and immunity of the influenzal infection and as the most important method of diagnosing influenza.

22. Enzymic Nature of Hemagglutination

Soon after the discovery of the hemagglutination phenomenon it was established that erythrocytes which react with the influenza virus completely or partially lose their capacity of being agglutinated after elution when the influenza virus of the same or another serologic type or other viruses possessing hemagglutinating properties similar to those of the influenza virus (mumps viruses, Newcastle disease viruses) are added. At the same time, the influenza virus eluted through the addition of fresh erythrocytes can react with them again (adsorption, agglutination, elution) (Hirst, 1942).

For the purpose of explaining these facts the hypothesis was expressed that the hemagglutination reaction is an enzymic process of reaction between the substrate -- the surface component of the erythrocyte stroma (receptor substances, receptors) -- and the enzyme, the receptolysin of the influenza virus.

The establishment of two facts were important for understanding the mechanism of reaction of the influenza virus with the erythrocytes: first, the discovery of an enzyme in cultures of the cholera vibrio, after the effect of which on erythrocytes the latter stop being agglutinated by influenza viruses (which destroy the receptor enzyme), and, secondly, the establishment of the phenomenon of nonspecific (that is, not by antibodies) depression of hemagglutination. A detailed study of these phenomena aided in a deeper understanding of the mechanism of reaction of influenza viruses not only with erythrocytes but also with susceptible tissue cells of the organism.

It seems expedient to analyze the principal facts pertaining to the reaction of the virus and erythrocytes in the hemagglutination reaction.

The least studied enzyme of the influenza viruses is the hypothetical receptolysin. Its properties may be judged by indirect data. It has been established that after heating the influenza virus to 56° an inactivation of the enzyme occurs, in connection with which the virus maintains its capacity of being adsorbed by erythrocytes and producing agglutination of them, but since the inactivated enzyme does not destroy the receptor substance of the erythrocytes elution of the virus does not occur. Burnet (1951)

suggested calling this state of the virus an indicator state, as has already been mentioned. If the agglutination of erythrocytes with heated influenza B virus occurs at room temperature the erythrocyte suspension may be stabilized again by the addition of diluted specific immune serum. If the agglutination of the chick erythrocytes occurs at 37° the virus becomes irreversibly bound to the erythrocytes and is not removed from the latter by the immune serum (Burnet, 1952). The heated virus may be removed from the erythrocytes also by means of treating the mixture with active (heated) virus (Hirst, 1949). According to Yeremeyev's data (1952), the influenza virus which is inactivated by heating, ultra-violet rays or moderate doses of formalin is eluted from the erythrocytes, although with greater difficulty than the natural virus; in concentrated (five-percent) sodium chloride solutions a complete elution of it from the erythrocytes occurs. According to the data of Hirst (1949), the heated virus bound to erythrocytes is not eluted from them in 5-10 percent sodium chloride solutions. In contrast to this, a virus treated with weak (from 1/40 to 1/200 N) solutions of calcium periodate is not eluted from the erythrocytes spontaneously or after the addition of the natural virus, but is eluted through the effect of 5-10 percent sodium chloride solutions.

Therefore, these experiments permit a double interpretation. The majority of investigators (Hirst, 1949; Tovarnitskiy, 1951; Burnet, 1954) consider these data to be evidence of the enzymic nature of the reaction of the influenza virus with the erythrocytes, whereas Yeremeyev (1952) believes that we are dealing with physico-chemical processes and denies the existence in the influenza virus of an enzymic capacity of destroying or altering some components of the surface of the erythrocytes responsible for virus adsorption. We shall return to a discussion of the problem of the nature of the reaction of the influenza virus and erythrocytes after an analysis of additional facts.

During recent years material has been accumulated which permits us to make judgments of the nature of the erythrocyte receptor substances.

By treating erythrocytes with calcium periodate, Hirst (1945) found that they stopped being agglutinated by the influenza virus. The addition of glucose neutralizes this periodate effect. Since the periodate acts on the carbohydrate groups of the mucoproteins and mucopolysaccharides, this gives us the grounds for considering mucin to be the erythrocyte substrate on which the virus acts. Burnet (1951) points out that N-acetyl-D-glucosamine is the active group of the substrate. The erythrocyte receptor

substance is resistant to heating up to 65°, and according to other data, up to 100° (Dreyzin, 1952). Treatment of the erythrocytes with formalin does not impair their capacity of adsorbing virus or being agglutinated and does not prevent the elution of the virus. On hemolysis of the erythrocytes in distilled water and by means of saponin the stroma of the erythrocytes maintains the capacity of adsorbing virus and does not prevent its elution. Oxidizers of the potassium permanganate and potassium dichromate type do not destroy the receptor substance. At the same time, tryptic digestion (effect on protein) and treatments with periodate (effect on polysaccharide) interfere with the erythrocyte property of adsorbing the influenza virus. In evaluating these facts, Tovarnitskiy (1951), in agreement with other authors, comes to the conclusion that the erythrocyte receptor substance belongs to the group of mucopolysaccharides.

Fractionation of the erythrocyte stroma has shown that the fraction rich in lipoids and containing carbohydrates contains the receptors for the influenza virus (substrate of the hemagglutinin effect) as well as the group antigens A and Rh. Afterwards, a protein-lipoid-carbohydrate complex was isolated which possessed these properties, while the ether-soluble residue did not have them (Howe, 1951).

Therefore, a study of the erythrocyte substrate with which the influenza virus reacts permits us to categorize it in the group of mucopolysaccharides or mucoproteins. The data presented speak on behalf of an enzymic rather than another type of reaction of the influenza virus with erythrocytes in the hemagglutination reaction.

The biological and antigenic properties of the substances which fix the influenza virus onto the erythrocytes were studied by Smorodintsev and Dreyzin (1953). These substances in the form of extracts were isolated from the erythrocytes of animals susceptible to the influenza virus (chickens, guinea pigs, mice), and were not isolated from erythrocytes of resistant animals (horses and dogs). When the influenza virus reacts with these substances they are destroyed.

Thereby, the treatment of the extracts with a single type of influenza virus (for example, A) is sufficient for them to stop agglutinating any influenza virus (A or B). Their antigenic properties are specific: sera obtained by means of immunization of animals with erythrocyte extracts and which are active with respect to them do not reduce the activity of erythrocyte extracts of other species of animals.

A receptor-destroying enzyme has been found in filtrates of cholera vibrio cultures by Stone (1947), Mulder and van der Veen (1948) and other authors. This enzyme acts both on the erythrocyte receptors and on the hemagglutination inhibitors. Isaacs and Bozzo (1951) showed that the receptor-destroying enzyme eliminates the nonspecific hemagglutination inhibitors in ferret sera. It is destroyed at 56° in 30 minutes, is adsorbed onto chick erythrocytes at 2°, and is eluted at 37°. Calcium ions exert a stabilizing effect on it.

Briody (1948) established the fact that the adsorption of influenza viruses depends on the same environmental conditions as the activity of the receptor-destroying enzyme. Both processes are depressed by oxalates, citrates and hexametaphosphates, and are accelerated by calcium and magnesium ions; fluorides and phosphates do not exert any influence on them.

In the reaction of a heated virus with erythrocytes with subsequent treatment of them with the receptor-destroying enzyme the virus becomes irreversibly bound to the erythrocytes without losing the capacity of reacting with antigens, fresh erythrocytes or inhibitor (Burnet, 1952).

Haga (1951) studied the effect of various cholera vibrio fractions. The polysaccharide fraction proved to be active; the fractions soluble in acetone, inactive.

Filtrates of the cholera vibrio, in destroying receptors and nonspecific inhibitors, do not act on the influenza antibodies contained in immune sera (Mulder, Nooijer, Brans, 1952).

Enzymes of this type have been obtained in other bacteria besides the cholera vibrio.

Fridman (1954) showed that in filtrates of two-day Mechnikov vibrio cultures a factor is contained which inactivates the nonspecific hemagglutination inhibitors and which does not affect the specific influenzal antibodies.

Filtrates of Clostridium perfringens cultures destroy the allantoic fluid inhibitors (Svedmyr, 1948).

At the present time, substances of the receptor-destroying enzyme type of cholera vibrios have been found not only in certain species of bacteria (the cholera-like vibrio, Clostridium perfringens) but also in snake venoms, bee toxins and in leeches. It may be considered proved that enzymes of the mucinase type constitute the effective principle of these preparations. After treatment of erythrocytes with them the latter lose their capacity of fixing the influenza virus. In their physico-chemical properties and stability these substances are similar to the receptolysin suggested for influenza viruses (Tovarnitskiy, 1951).

All these facts are supplementary, even though indirect proofs of the enzymic nature of the reaction of influenza viruses with erythrocytes.

Changes in Erythrocytes After the Effect of Influenza Viruses and Receptor-Destroying Enzymes on Them. As has been mentioned above, following treatment with influenza viruses or filtrates of cholera vibrio cultures erythrocytes lose their capacity of readsorbing influenza viruses and other viruses possessing a similar hemagglutinin. Thereby, the electrophoretic mobility of the erythrocytes is sharply reduced also, particularly after treatment of them with the receptor-destroying enzyme (Ada and Stone, 1950).

The influence of the influenza virus on the electrokinetic potential of erythrocytes has been studied by Sereda and Yeremeyev. Sereda (1952) showed that the influenza virus reduces the electrokinetic potential of guinea pig, human, and chicken erythrocytes but not of horse erythrocytes, that is, only of those animals the erythrocytes of which are agglutinated by influenza viruses. The rate of decrease depends on the temperature and is best expressed at 37°. The decrease in the electrokinetic potential occurs from a dose of the virus which is three or four times smaller than the minimum hemagglutinating dose.

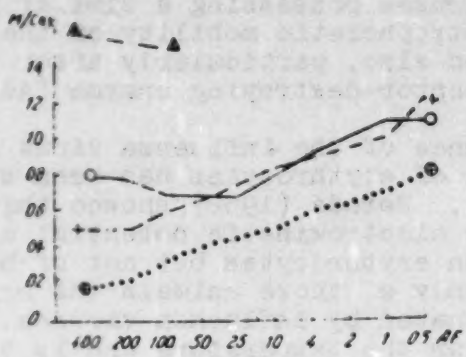
Yeremeyev has obtained similar data. In his experiments the rate of movement of erythrocytes in a Northrop-Abramson chamber was slowed up even after treatment with 0.05 of an agglutinating dose of the virus. The larger the quantity of virus with which the erythrocytes were saturated the more the rate of movement of them was slowed up or the more their electrokinetic potential decreases. After treatment of erythrocytes with an immune serum specific for them the rate of movement of them also decreased (Fig. 60).

During the adsorption phase of the virus the erythrocytes are more resistant to lysis with sodium choleate; after elution this resistance becomes normal (Bateman, Davis, McCoffrey).

After treatment of the erythrocytes with influenza virus or receptor-destroying enzyme the antigenic composition of the erythrocytes is changed, which is evidently associated with a change in the spatial configuration of their antigens (Stewart and Quilligan, 1951).

In connection with this, it should be noted that after treatment with the influenza viruses or receptor-destroying enzyme the erythrocytes acquire the property of being agglutinated by low dilutions of any sera (Burnet, McCrea and Stone, 1946). This phenomenon apparently does

not depend on the presence of panagglutinins in the sera, because the agglutination of such erythrocytes occurs even after the addition of mucoproteins and Congo-red dye to them (Burnet, 1952). Apparently, we are dealing here with a change in the electric charge of the surface of the erythrocytes, in connection with which their resistance in suspensions decreases markedly.



- Rate of Movement of Erythrocytes with Antibodies
+ Same with influenza virus
○ Same after being kept at 37° for four hours
▲ Without the virus

Fig. 60. Rate of Movement of Erythrocytes Loaded with Influenza Virus and Antibodies in an Electric Field (Yeremeyev, 1953).

These changes in the erythrocytes were obtained in in vitro experiments. However, similar changes apparently can occur in vivo, which is evident from the observations of Svet-Moldavskiy and Lozovskaya (1952). After parenteral injection of the influenza virus into chickens and guinea pigs and the taking of blood between the second and tenth day after infection the erythrocytes possess a decreased capacity of being agglutinated by influenza viruses, which the authors explain by the traumatic effect of the virus on them.

23. Nonspecific Hemagglutination Inhibitors

In various substrates nonspecific hemagglutination were found.

First of all, they have been found in human sera and animal sera. They are encountered in particularly high

titers in the sera of ferrets (Burnet and McCrea, 1936) and rabbits (McCrea, 1946), and, on the other hand, in low titers in the sera of guinea pigs and chickens (Gorbunova, 1953), but the inhibitors are found to varying degrees in the sera of any species of animals as well as in human sera.

Contradictory data were originally obtained by various investigators concerning the nature of these inhibitors and the character of their effecton the influenza viruses. Thus, Smorodintsev and Shishkina (1951) showed that normal sera of guinea pigs, white rats, chickens and other animals contained thermolabile substances which neutralize the infectious and hemagglutinating properties of the influenza virus. This factor does not exist in organs free of blood; it is not identical with complement or with enzymes. Heating of the serum at 56° for 30 minutes destroys this factor. Undiluted sera of guinea pigs, chickens and rats neutralize more than 100,000 LD of the virus for mice; the corresponding values for the sera of white mice and horses are equal to 10 and 100; the sera of rabbits and sheep occupy an intermediate position. In these experiments attention should be directed to the thermolability of the inhibitors and their capacity of neutralizing not only hemagglutinating but also infectious properties. On the other hand, there is an abundance of data according to which the inhibitors of normal sera do not possess virus-neutralizing activity but only inhibit hemagglutination, thereby being more thermostable (see the works of Burnet and McCrea mentioned above). Apparently, contradictory results obtained by various investigators depended on the characteristics of the methods of investigation used, the various types of influenza viruses on which the effect of the inhibitors was tested as well as on the fact that the investigators were dealing with inhibitors of different natures.

At the present time, two types of thermolabile inhibitors are known in the sera of animals: one of them has been found by Francis; the other, by Chu.

Francis (1947) found that the influenza B viruses after heating to 56° for 30 minutes and while preserving their hemagglutinating properties cannot be used as an antigen for combining with the specific antibodies of human sera. The hemagglutinating properties of this virus are markedly depressed by sera, whereby the hemagglutination depression does not occur because of the antibodies but rather on the strength of the normal constituents of the serum. For example, in one of the experiments paired sera of an influenza patient gave hemagglutination inhibition titers of 1:28 and 1:512 with the natural virus, and in a

reaction with the heated virus both sera suppressed the hemagglutination in a titer of 1:4096.

Chu (1951) studied the effect of the inhibitor of normal mouse sera. The same strains of influenza virus adapted to various hosts possess different degrees of sensitivity to the inhibitor: the hemagglutination of allantoic strains is depressed to a greater degree than that of strains adapted to mice. The inhibitor is destroyed at 56° in 30 minutes, is adsorbed by kaolin, and requires the presence of calcium ions for the manifestation of its activity. Calcium periodate in a concentration of 0.05 M does not destroy it. The inhibitor is destroyed by the effect of an unpurified filtrate of the cholera vibrio and is not destroyed by the purified receptor-destroying enzyme or trypsin.

Therefore, the Francis inhibitor suppresses hemagglutination, and its titer is increased markedly with heating of the virus; it is destroyed by calcium periodate, an unpurified filtrate of cholera vibrio and a purified preparation of the receptor-destroying enzyme. The Chu inhibitor is more active with respect to the allantoic strains than those adapted to mice (equally with respect to heated and natural strains), is not destroyed by calcium periodate or purified receptor-destroying enzyme, is inactivated by unpurified cholera vibrio filtrates. Both inhibitors are thermolabile.

The inhibitor α (Francis) does not neutralize the infectious properties of the virus, is more thermostable, is destroyed by the active virus and by filtrates of the cholera vibrio or by a purified preparation of the receptor-destroying enzyme obtained from these filtrates. Inhibitor β (Chu) can neutralize the influenza virus, is more thermolabile, is not destroyed by the active virus or by the receptor-destroying enzyme, but is inactivated by crystalline trypsin (Sampaio and Isaacs, 1953). Tyrell (1954) used the method of electrophoresis in starch for the dilution of both inhibitors and antibodies.

In the fractionation of proteins the antibodies were found to be bound to γ-globulin; the α-inhibitor, with α-globulin; the β-inhibitor, with β-globulin.

Smith and Westwood (1950) showed that the Francis phenomenon in rabbits occurs to the same degree at temperatures of 2° and 25°.

Sampaio (1952) found the Francis inhibitor in a large quantity in rabbit and guinea pig sera; it was absent from the sera of mice and ferrets. The Chu inhibitor was found in the sera of rabbits, guinea pigs, ferrets and mice.

Serum inhibitors act unequally on the various virus

strains.

Thus, Hilleman and Warner (1953) found inhibitors in human sera which acted on the A and B viruses but not on the C virus. Their titer with respect to the WS strain was no greater than 1:10; with the PR8 strain, less than 1:320. In other series of sera inhibitors were found active with respect to the PR8 and FM strains but not to the Lee and FW1-50 strains.

Experiments with the partial purification of these inhibitors indicate a similarity of many properties of serum inhibitors to inhibitors found in other tissues and to erythrocyte receptors (Hirst, 1949).

The mechanism of destruction of the inhibitors by the influenza virus enzyme (receptolysin) is the same as the mechanism of destruction of the erythrocyte receptors (Tovarnitskiy, 1951).

The influenza virus destroys the inhibitor of the hemagglutination of substances in the O and A blood groups as well as the gonadotropic hormone. All these substances are mucoids containing acetylglucosamine (Whitten, 1948).

Aside from the inhibitors of mucoid-polysaccharide origin similar to the erythrocyte receptors, other nonspecific inhibitors, the nature of which is still unclear, are found in human and animal sera. Thus, Utz (1949) isolated a lecithin-like substance which inhibits hemagglutination from the serum of animals (as well as from the internal organs).

Thermostable inhibitors which depress hemagglutination have been found by Fridman (1952) in normal horse, rat, sheep and human sera. These inhibitors are not destroyed after heating the sera or even after autoclaving them. The author believes that this nonspecific inhibitor acts on the nonspecific spontaneous hemagglutination.

The inhibitor of the Francis type has been found in the sputum of patients with chronic bronchitis (Curtain, Marmion, Pye, 1953). The purified preparation which inhibits the hemagglutination of natural viruses (A, A-prime and B) in titers of 80-1000 and heated viruses in titers of 4000-9000, was destroyed through the effect of the receptor-destroying enzyme.

A thermostable inhibitor was found in the saliva of people (Seltsam, Lanni, Beard, 1949). This inhibitor is destroyed at 100°; its titer is higher when the reaction is performed with heated than with the natural virus; the latter destroys the inhibitor (therefore, it is partially similar to the α-type inhibitor).

More of it is contained in the secretion of the submaxillary and sublingual glands, which are rich in mucoids, than in the secretion of the parotid glands.

A nonspecific inhibitor of the salivary glands of sheep was studied in detail (McCrea, 1953). According to the nature of the effect on the influenza virus it can be classified among the α-type inhibitors. According to its chemical nature this inhibitor is a mucoid with a molecular weight of about 87,000; it is destroyed by the active A and B viruses and the receptor-destroying enzyme of the cholera vibrio as well as by calcium metaperiodate.

An inhibitor from the urine of healthy people which reacts with viruses of influenza, mumps and Newcastle disease, has been isolated and purified. It has been shown to be a mucoprotein with a molecular weight of $7.0 \cdot 10^6$; it has straight-chain molecules with an axial index of about 100. Even 0.0003 milligrams of the preparation produced a notable hemagglutination inhibition. The inhibitor stopped reacting after its reaction with the influenza virus (Tamm and Horsfall, 1952).

Hemagglutination inhibitors have also been found in cow's milk (Lepine, Lepine and Beard, 1949). They have a more active effect on the heated virus than on the natural virus and possess a moderate thermostability, withstanding pasteruization.

An inhibitor in human meconium has been isolated and purified (Curtain, French and Pot, 1953). It proved to be relatively thermostable (it withstood a one-minute heating to 100° at a pH of from 3 to 11). On incubation with various influenza virus strains or with the receptor-destroying enzyme its activity decreased. The inhibitor proved to be resistant to the effect of trypsin and taka-diastase.

The inhibitors of the hen's egg have been studied in particular detail. Three mucopolysaccharides are contained in the albumen of the hen's egg: an ovomucoid -- a lysozyme substrate; an ovomucoid which is a trypsin inhibitor; an ovomucin, which is an influenza virus inhibitor (Nigul', 1952). The latter was found by Lanni and Beard (1948) and was studied in detail by Gottschalk and Lind, (1949); it is contained also in allantoic fluid (Hardy and Horsfall, 1948). On electrophoretic analysis, three components are found in ovomucin; the polysaccharide contains glucose, mannose and hexosamine (Neuműller, 1953).

A quite satisfactory purification of the egg albumen inhibitor is obtained by filtration of the latter through a special kind of filter paper: subsequent extraction of the sediment on the paper produces a preparation which is 80 times more active than the original (Csaky, Lanni and Beard, 1950). The egg inhibitor constitutes no more than 10 percent of the ovomucin (Fazekas de St. Groth and Gottschalk, 1951).

The inhibitor consists of quite large particles the

sedimentation constant of which is about 200 S. It does not pass through graduated colloid membranes having pores 125 millimu in size (Svedmyr, 1948).

Purified preparations have been obtained having an activity of four hemagglutinating units in 0.08 γ; in the electron microscope the inhibitor looks like a network of fibers; the virus particles in this network are represented by aggregates (Kozinski and Mikulaczek, 1915). After the reaction with the influenza virus the ovomucin is destroyed in proportion to the concentration of the components and to the temperature within limits of 4-37° (Nigul', 1952).

The protein inhibitor suppresses hemagglutination which is caused by the active virus in much lower titers (1:50-1:100) than that produced by heated or formalinized virus (1:54,000-1:170,000). This is apparently explained by the enzymic activity of the live virus, which destroys the inhibitor (Lanni and Beard, 1948).

The egg-white inhibitor is not identical with the serum inhibitor. On comparison of different strains of influenza virus some are found to be more sensitive to the egg-albumen inhibitor; others, to the normal rabbit serum inhibitor. After heating to 56° all the strains are more sensitive to the egg white inhibitor, and after heating to 61° their sensitivity to the rabbit serum inhibitor is increased (Bernkopf, 1949). The allantoic fluid inhibitor is inactivated by periodate and only partly by proteolytic enzymes -- trypsin and phytin (Svedmyr, 1949).

Up to this time we have dealt with egg-white and allantoic fluid inhibitors. However, in addition to them, there is an inhibitor in the chorioallantoic membrane which is different from those mentioned. It is apparently not found on the surface but rather inside the cells, and is only weakly affected by the receptor-destroying enzyme, withstanding boiling for two minutes and is destroyed by the virus during the course of its multiplication (Edney and Isaacs, 1950).

Hemagglutination inhibitors were also found in bacteria. Kozinski and Mikulaczek (1951) showed that autolysates of S6 and 155 strains of typhoid bacilli inhibit hemagglutination; the Vi-strains of 901-H and 901-O typhoid bacillus, proteus, colon bacillus and dysentery microbes do not possess this property. Of the three fractions of typhoid microbes -- the polysaccharide-protein, the polysaccharide, and the nucleoprotein fractions -- the first two are active, and the third one is active to a slight degree. The first two fractions are thermostable; the third one loses its inhibitory activity after being heated at 100° for 30 minutes. Inhibition of hemagglutination is also accomplished by the polysaccharides of the capsulated

bacteria (Klebsiella aerogenes, Klebsiella cloacae) without exerting any effect on the biological activity of the influenza virus (MacPherson, 1953).

Extensive investigations of mucins and mucoids found in various substrates have been carried out by Anderson and co-workers (1948). In human serum they found two inhibitors aside from the antibodies: a nonspecific inhibitor which inhibits hemagglutination of the heated virus, and the Francis inhibitor which inhibits hemagglutination of the heated B (Lee) virus. The titer of the latter is higher than that of the former; both are inactivated by the receptor-destroying enzyme. The Francis inhibitor is in the globulin fraction. Heating of the serum in a neutral reaction increases its titer; in an acid or alkaline reaction it is destroyed. The nonspecific inhibitor is destroyed by heating at a pH = 7-8. The Francis inhibitor is closely connected with the serum mucoid (mucoprotein). Proteolytic enzymes and periodate destroy the Francis inhibitor and the nonspecific inhibitor; the latter, less actively. The inhibitor of the Francis type is also found in the mucins of ovarian cysts and in the substance of blood group O. Small doses of the periodate are not destroyed but rather increase the activity of the Francis inhibitor from the ovarian cysts. This inhibitor is destroyed by enzymes of active influenza viruses.

Therefore, in animal tissues a considerable quantity of substances has been found which inhibit the agglutination of erythrocytes by influenza viruses. Despite the differences in the properties of a number of these substances the preparations studied have been shown to be mucoproteins, and the reaction of them with influenza viruses is in general similar to the reaction of the latter with erythrocyte receptors.

24. Theories Explaining the Hemagglutination Mechanism

The majority of modern investigators regards the agglutination reaction from the standpoint of the hypothesis presented above.

Thus, Tovarnitskiy and Lutikova (1949) point out that after adsorption of the virus onto erythrocytes hydrolysis of the receptor mucoid of the erythrocytes by the virus enzyme occurs, as a result of which an elution of the virus from the erythrocytes occurs. After heating the virus to 56° the enzyme of the virus (receptolysin) is destroyed; therefore, no elution of the virus occurs from the erythrocytes. The destruction of the erythrocytes may be achieved by enzymes of the mucinase, lecithinase or hyaluronidase type; after this, the erythrocytes do not adsorb

the influenza virus. The forces which control the processes mentioned above are intermolecular in their nature.

Burnet (1951) notes that after heating the virus at 56° it is found in an "indicator state" in which its hemagglutinating properties may be suppressed by various mucoid substances which destroy the live virus. Mucoid inhibitors and erythrocyte receptors can be inactivated by the receptor-destroying enzyme of the mucinase type which exists in filtrates of cholera vibrio cultures. Erythrocytes treated in this way are not agglutinated by the virus, and the tissues become insensitive to infection.

The majority of investigators in the Soviet Union adheres to the point of view mentioned (Smorodintsev and Dreyzin, 1953; Romanenko and Khayt, 1953), as do the majority of investigators abroad (Hirst, 1942; Magnus, 1954; and others).

Yeremeyev (1949) takes a particular point of view of the hemagglutination mechanism. He believes that the adsorption and elution of the influenza virus from the erythrocytes constitute a physico-chemical process which is not associated with the simultaneous enzymic destruction of erythrocyte receptors. In this work he has shown that erythrocytes placed in five-percent sodium chloride solution at 37° are rapidly eluted from the virus after adsorption of it, thereby preserving the capacity of adsorbing the influenza virus again. This does not occur when the reaction is performed in physiological solutions or in a phosphate buffer. In the subsequent works of Yeremeyev (1952, 1953) his views are opposed to the views of Hirst, denying the existence of enzymes in the influenza viruses and explaining the phases observed exclusively by physico-chemical surface processes.

The enzymic theory of reaction of the influenza virus with erythrocytes in the hemagglutination reaction seems to us to have a sounder basis. It is indisputable that the reaction between the influenza virus and erythrocytes is a very complex process in which physico-chemical phenomena and enzymic processes participate. The adsorption of the virus onto erythrocytes and elution -- the initial and final stages of this process -- represent the result of the reaction of surface forces of the virus particle and the erythrocytes; apparently, it explains also such phenomena as the agglutination of erythrocytes and the change in their electrokinetic potential. At the same time, a multitude of facts indicates that after adsorption of the virus particles by erythrocytes the former show enzymic activity the essence of which is a destruction of the mucoids located at the surface of the cell. There is no point in relisting the numerous facts which prove the

enzymic nature of this reaction and the existence of enzyme of the mucinase type in influenza viruses. A close acquaintance with the material presented above not only proves the correctness of the enzymic theory but also makes it possible to elucidate many characteristic features of the enzymic processes which develop in the reaction of influenza viruses with erythrocytes.

Attempts to give the quantitative characteristics of the reaction between the virus and erythrocytes have been undertaken by a number of authors. Such attempts were made soon after the discovery of the hemagglutination phenomenon. Thus, it was calculated that under ordinary conditions of performing the reaction the latter occurs in the presence of 10^4 - 10^5 virus particles. The ratio of the number of erythrocytes to the number of virus particles at the end-point of titration is equal to approximately unity (Friedewald and Pickels, 1944).

Of the later works the investigations of Lanni and Sagik deserve attention.

Lanni and Lanni (1952) proposed the quantitative theory based on the following considerations. The virus particle is bound to the receptors of two different erythrocytes; one molecule of the inhibitor neutralizes the hemagglutination of the virus particles; the reaction between the virus and the inhibitor or erythrocyte is irreversible and does not reach an equilibrium during the usual time for performing the experiment. While we shall omit the course of his reasoning, we should like to present certain equations which characterize the quantitative aspect of the hemagglutination reaction. The quantity of free virus is determined by the equation:

$$V = V_0 e - k_1 R_0 t, \qquad (1)$$

where e is the natural logarithm base, V_0 and V are the initial and final concentration of free virus, R_0 is the initial concentration of erythrocytes, k_1 is the adsorption constant of the virus onto erythrocytes, and t is the time.

The number of virus particles, each of which agglutinated a pair of erythrocytes, is determined by the equation:

$$VR_2 = \frac{V_0}{(1 + k_3 J_0 / k_1 R_0)^2}, \qquad (2)$$

where VR_2 is the concentration of virus sought; J_0 is the initial concentration of inhibitor; k_3 is the adsorption constant of the virus onto the inhibitor.

Based on the reaction kinetics equations and measure-

ments made, the molecular weight of the inhibitor is of the order of $8 \cdot 10^6$.

Sagik and co-authors (1954), on the basis of experiments and calculations have come to the conclusion that each chick or human erythrocyte has about 300 places onto which the influenza virus particles may attach themselves, which correspond to approximately two percent of the erythrocyte surface. Practically all erythrocyte receptors are destroyed before the elution of the virus begins. Spontaneous elution at 37° for 24 hours requires a threshold quantity of no less than three but less than five particles of virus per cell.

At the present time it seems to be difficult to give an evaluation of the phenomena of the reaction between the influenza virus and erythrocytes in the hemagglutination reaction by a mathematical analysis. They should be welcomed as attempts to go from a description of the qualitative rules and regulations of the process to an understanding of the quantitative interrelationships. At the same time, it cannot be overlooked that the simplifications made by authors not uncommonly lead them to erroneous conclusions. This applies specifically to the work of Sagik and co-authors; the conclusion which they drew as to the presence of a quite limited portion of the erythrocyte surface and of the few areas of which can fix the influenza virus contradicts the existing facts, particularly the electron microscopic observations. It is sufficient to compare the microphotographs obtained by Draganov (Figs. 52-55) as well as the even more demonstrative data (Fig. 61) of Vieuchange and Gentini (1954) in order to understand how hasty the conclusions and how unsound the quantitative calculations of Sagik and his co-authors were. Here, it is very fitting to recall the investigations of Romanenko and Khayt (1953), who showed that even with the same ingredients and the same concentration of them the results may vary considerably depending on the sequence and the times of mixing the components.

The hemagglutination reaction discovered and the study of their mechanism constituted an important phase of the development of our knowledge not only of influenza viruses but also in the field of general virological problems. Many rules and regulations shown during the course of study of this reaction have proved to be applicable to the interpretation of processes of reaction of the viruses with susceptible tissues, which is of particular importance for understanding the pathogenesis of influenza. At the same time, the hemagglutination reaction as a method of investigation has become widespread in the study of the antigenic structure of influenze viruses, their variability as

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well as in the study of immunity and epidemiology of influenza.

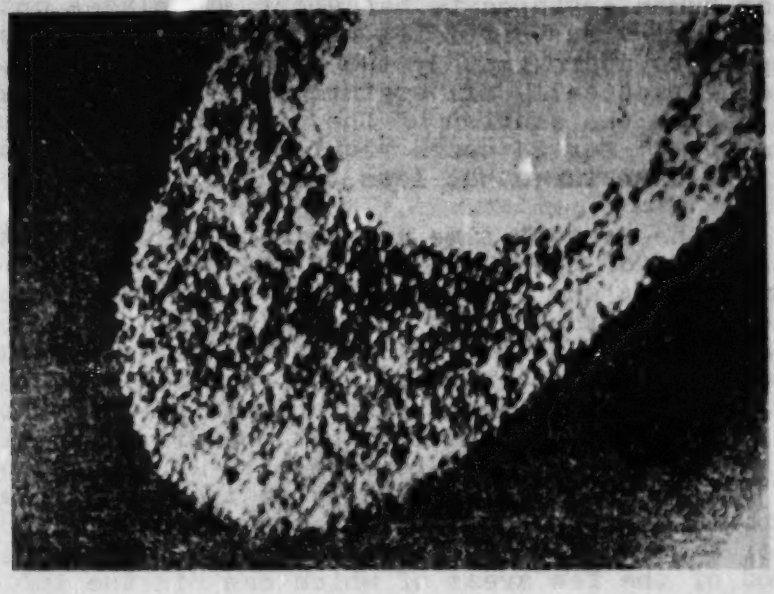

Fig. 61. Influenza A (PR8) Virus Adsorbed Onto Stroma of Chick Erythrocyte (Vieuchange and Gentini, 1954).

25. Antigenic Structure. Soluble Antigen and Hemagglutinin

Virus suspensions contain two components: elementary bodies and a soluble antigen. These components may be divided by means of fractional centrifugation, because they are of different sizes, and therefore they precipitate differently. The sedimentation constant of elementary bodies is equal to about 600 S, which corresponds to a particle diameter of 80-120 millimu; the soluble antigen has a sedimentation constant of about 30 S, which corresponds to a diameter of 10-15 millimu. These data, obtained first by Hoyle and Fairbrother (1937), were confirmed by the verification investigations of Lennette and Horsfall (1941), Friedewald (1943), Henle and Wiener (1944), Stanley (1944), Wiener, Henle and Henle (1946), Peterson and Yeremeyev (1948), Yeremeyev and Orlova (1949), Hoyle (1950, 1952), Ada, Perry and Pye (1953) and other authors.

In accordance with these data the soluble antigen

is not bound to the virus particle, is not adsorbed by erythrocytes, does not produce hemagglutination of the erythrocytes and can be found by means of the complement-fixation reaction. In allantoic cultures it is found in lesser quantities than in the lungs of mice infected with influenza. However, its quantity increases markedly when the influenza virus is destroyed by ultrasound.

The soluble antigen reacts with immune sera in lower titers than the elementary virus bodies. The antigen of the elementary bodies, which bind the complement, is different from the soluble antigen. The soluble antigen is type-specific within the limits of the types of influenza pathogens (A and B respectively) and does not possess any strain specificity.

Antibodies are formed to the soluble antigen during the course of infection but not of immunization (formalin-killed virus). When the influenza virus is treated with ether and subsequent adsorption onto erythrocytes in the preparations it is possible to distinguish two fractions -- the hemagglutinin and the soluble antigen. The latter is a ribonucleoprotein. This has given Hoyle (1952) the grounds for considering it an inner constituent of the influenza virus.

According to the data of Peterson and Yeremeyev (1948), the soluble antigen is very stable: when kept under thermostat conditions (37°) for 10 days it comes down in the precipitate, but its antigenic properties are not thereby reduced in the complement-fixation reaction; at a temperature of 2° they are unchanged for more than a month. After repeated freezing and thawing of virus suspensions their activity in the complement-fixation reaction increases. Heating for an hour at a temperature of 50-55° does not reduce the antigenic activity of the soluble antigen. It is reduced at 60-70° after 30 minutes and is immediately destroyed at 100°. The activity of the soluble antigen is preserved in the pH zone from 6-10; in the acid zone it comes down to the form of an insoluble precipitate (pH = 3.5-4.5). In half-saturated solutions of ammonium sulfate a precipitation of soluble antigen occurs. Acetone and ether partially denature it. By the Crediner method it is possible to obtain an active fraction of the soluble antigen; a carbohydrate-protein fraction active in the complement-fixation reaction may be also obtained by the method of Boisvain. However, these fractions, like the virus treated with urea, possess insignificant immunogenic properties with respect to the active virus (Yeremeyev and Orlova, 1949).

Recently, attempts have beem made at studying the soluble antigen with the electron microscope. Hoyle and

others (1953) showed that in natural preparations of the influenza virus particles 1250 Å in size predominate, and there is a small number of particles 100-500 Å in size. After shaking in ether only particles 100-500 Å remain. By means of adsorption onto erythrocytes the author divided the virus preparations into two components. One of them is adsorbed onto erythrocytes, is 120-500 Å in size and, in the author's opinion, is a hemagglutinin; the other is not absorbed onto erythrocytes, has a size 120 Å, is active in the complement-fixation reaction and should be considered the soluble antigen. Therefore, in the author's opinion, the elementary particle of the virus consists of two components: the soluble antigen and the hemagglutinin.

Similar data have been obtained by Tyrell and Horsfall (1954) who destroyed the influenza viruses (A, A-prime and B) by freezing and thawing or with concentrated solutions of urea with subsequent centrifugation. After such treatment a destruction of the virus particle into small fragments of almost the same size occurred along with which amorphous masses were found in the preparation (Fig. 62). The latter, apparently, represent the hemagglutinin. The authors come to the conclusion that the soluble antigen is a protein and constitutes the main antigenic material of the virus.

Certain authors (Magnus, 1954) believe that in contrast to the soluble antigen the second component is not separable from the elementary bodies, has the same sedimentation constant as they do, is adsorbed onto erythrocytes causing their agglutination; the complement-fixation reaction titers with it correspond to hemagglutination reaction titers, and in both reactions it shows a strain specificity, that is, the second component is identical with the elementary bodies of the virus.

In Table 22 the main properties of both antigens are summarized.

In the evaluation of the data of this Table it is necessary to take into consideration the fact that the first component here is identical with the elementary bodies and is in contradistinction to the soluble antigen; this is not altogether true, because the soluble antigen also is included among the elementary bodies.

Above, it has been mentioned that the hemagglutinin can be separated from the virus particle, which in this case does not have any hemagglutinating properties. However, since the properties of the hemagglutinin, isolated from the virus particle, have practically not been studied it is necessary to compare the properties of the whole virus particles (elementary bodies) and their parts (soluble anti-

gen). Although the soluble antigen was discovered quite long ago it has been inadequately studied as yet. Its biological importance will be discussed in the chapter on the pathogenesis of influenza. Here it is fitting to present certain considerations on the structure of elementary bodies of influenza viruses.

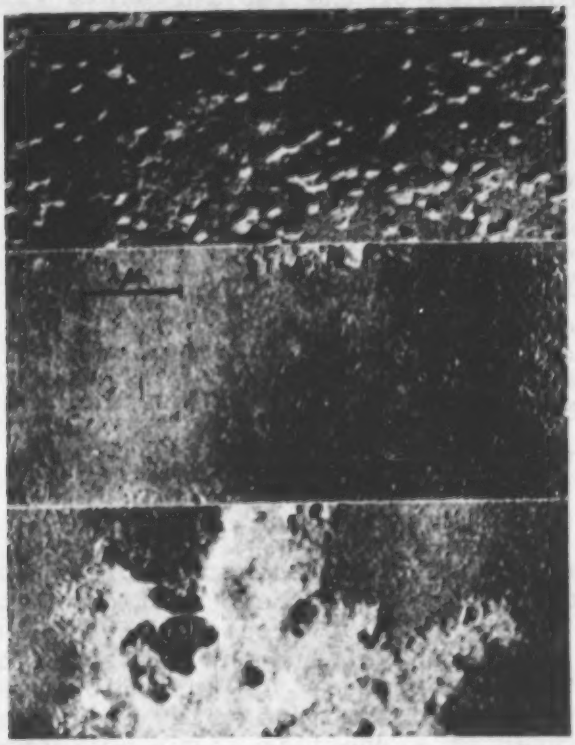

Fig. 62. The Effect of Repeated Freezing and Thawing on the Influenza A (PR8) Virus.

a -- partially purified virus preparation; b -- the same preparation after freezing and thawing 50 times; c -- amorphous mass of the same preparation (Tyrell and Horsfall, 1954).

Recently, a progressively greater number of facts have been accumulating which make it possible to reject the previous concepts of the virus particle as a simple structureless clump of protein (nucleoprotein). New data indicate a quite complex and distinct structure of the virus

elementary bodies. An analysis of factual material pertaining to this problem would lead us away from the principal content of this book, and therefore we are limiting ourselves to a brief description of certain data and generalizations given in the review of Ryzhkov (1954).

Table 22

Principal Properties of the Two Influenza Virus Antigens

Properties	Virus Particle	Soluble Antigen
Size in millimu	80–120	10–15 or 15–25
Sedimentation constant in S	600	30
Adsorption onto erythrocytes	+	–
Hemagglutination	+	–
Destruction of receptors and inhibitors	+	–
Toxic properties	+	+
Infectious activity	+	–
Interference	+	–
Complement-fixation reaction	+	+
Specificity of antigen	Strain	Species

At the present time, the structure of the bacterial viruses (bacteriophages) has been most completely studied. On the basis of the data of electron microscopy, study of the reaction of the phage with the bacterial cell and the biochemistry of this process the bacteriophage particle appears to be a complex structure (Fig. 63). The basis of it is constituted by a specific virus nucleoprotein, the carrier of species and individual properties of the given phage. The nucleoprotein is included in a protein membrane, devoid of nucleic acids, which serves as a protection for the virus against harmful external influences. The majority of the phages has the shape of a sphere with a process by which the phage particle is fixed to the surface of the bacterial cell. The phage particle as a whole does not penetrate into the bacterial cell: after fixation and apparently the impairment of the integrity of the bacterial membrane the nucleoprotein is poured into the bacterial cell, and the membrane of the phage remains outside. During the course of intracellular multiplication it is impossible to detect the phage particles by morphologic methods in the bacterial cell; however, through chemical analysis

nucleic acids inherent in the phage nucleoprotein and absent in normal bacterial cells are always found. There are grounds for the belief that the phage nucleoprotein is made of comparatively simple compounds of the amino-acid type and purine bases. Developed phage particles can be found at the end of its development cycle in the bacterial cell. If the cell is destroyed shortly before the beginning of lysis, empty phage membranes are found in it. Under ordinary conditions lysis of the bacterial cell is accompanied by the exit of completely formed phage particles containing nucleoprotein and enclosed in the protein membrane from it. Observations of incomplete or defective forms of viruses (more will be said about the incomplete forms of influenza virus in the chapter on pathogenesis) permit us to conclude that many viruses have a structure similar to the structure of bacteriophages. In evaluating these facts, Ryzhkov points out that the elementary virus bodies resemble spores or cysts. The presence of a membrane explains the enzymic inertia of the viruses in vitro in contrast to the high degree of activity of them in vivo where the virus nucleoprotein reacts with the cell cytoplasm. The presence of the membrane also explains the incomplete inactivation of the virus by various substances which block the membrane but which do not act on the nucleoproteins (antibodies, heavy metal salts in low concentrations, proteins which make up the virus, protein complexes, etc.).

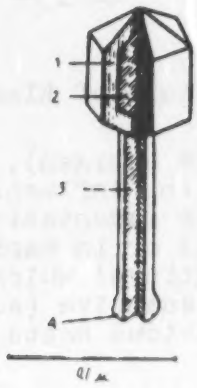

Fig. 63. Schematic Representation of Phage Particle (Anderson and Others, 1953).

1 -- solid membrane 125 Å in thickness; 2 -- nucleoprotein and water; 3 -- hollow tail; 4 -- thickening on the tail -- the site of attachment to the host cell.

This hypothesis makes it possible to explain the data presented above concerning the soluble antigen and the hemagglutinin and gives us the grounds for expressing certain considerations on the structure of elementary influenza virus bodies (Fig. 64). It may be assumed that the soluble antigen, a nucleoprotein, is the carrier of the type properties of the influenza virus. It is enclosed in a protein membrane, which, however, is not inert but rather possesses enzymic functions (mucinase) producing the phenomenon of erythrocyte hemagglutination. The significance of the hemagglutinin as a substance providing for the possibility of penetration of the influenza virus into susceptible cells will be discussed in the chapter on pathogenesis. Here we should like to note that this hypothesis, which we were the first to propound in 1950 (Zhdanov, 1950), is in good agreement both with the material and generalizations made in the work of Ryzhkov mentioned and with the current data concerning the nature of the soluble antigen and the hemagglutinin.

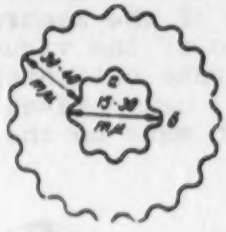

Fig. 64. Diagram of Structure of Elementary Body of Influenza Virus.

a -- nucleoprotein (soluble antigen), which is the carrier of the type properties of the influenza virus (types A, B) and the adaptive properties (adaptation to metabolism in the bodies of various hosts or in various tissues); b -- protein membrane (hemagglutinin) which possesses enzymic (mucinase, peptidase) and adaptive (adaptation to destruction of mucoproteins of various hosts or various tissues) properties.

The entire subsequent presentation of the data concerning the antigenic structure of influenza viruses is based on the study of the second component of the virus preparations, that is, of the virus particles (elementary bodies of influenza virus).

26. Methods of Study of Antigenic Structure

For the purpose of solving the problem of the serologic classification of influenza virus various methods of investigation have been used.

In this review those methods are being presented chiefly which we studied experimentally afterwards. We are dwelling chiefly on a description of the neutralization reaction of the pathogenic properties of the virus in experiments on mice and on the chorioallantoic membrane of chick embryos, the hemagglutination inhibition reaction in vitro, the neutralization reaction of hemagglutinins in the allantoic cavity of chick embryos, the complement-fixation reaction and the reactions of immune sera absorption.

The Reaction of Neutralization of Pathogenic Virus Properties. Initially after the discovery of the influenza virus serologic methods of identification amounted to neutralization experiments on mice. A detailed description of the neutralization reaction has been presented in the textbooks of Solov'yev (1948), Shubladze and Gaydamovich (1949). Here we should like to note that for the purpose of determing the degree of neutralization of the pathogenic properties of a given type of virus by the corresponding antiserum various mixtures of the virus with the serum are prepared in vitro and introduced into mice intranasally under light ether anesthesia.

The works of Smith and Andrewes (1938) and also of Magill and Francis (1938) on the serological typing of different influenza virus strains were carried out on the basis of neutralization experiments. However, this method is imperfect as a result of the fact that the factor of different degrees of pathogenicity of the serologically studied strains for mice is involved in the experiment, despite the fact that all the influenza virus strains studied at that time were adapted to mice.

In the experiments of Gorbunova (1953) this method produced satisfactory results with s rains which were well adapted to mice. In Table 23 data are presented on the neutralization reaction in mice on viruses A (PR8), A-prime (YeM-double prime) and B (Lee) with immune rat serum to the A (PR8) virus.

As seen from Table 23, A (PR8) serum neutralizes no less than 1000 doses of this virus, exerting a hardly noticeable effect on the A-prime and B viruses. The increase in the quantity of virus of over 1000 LD_{50} in the experiment usually leads to indistinct results, because this quantity is apparently the limit for the given method.

Table 23

Data of Neutralization Reaction of Influenza Virus by Immune Sera in Experiments on Mice

Virus			Serum		No. of Mice	Observation for 10 days	
Type	Strain	LD_{50}	Against Strain	Dilution		Lived	Died (Pneumonia)
A	PR8	1	PR8	1:10	5	5	0
		10			5	5	0
		100			5	5	0
		1 000			5	5	0
A_1	E.M.	1	PR 8	1:10	5	3	2
		10			5	1	4
		100			5	0	5
		1 000			5	0	5
B	Lee	1	PR8	1:10	5	2	3
		10			5	0	5
		100			5	0	5
		1 000			5	0	5

With the transition to the technique of isolating the strains on chick embryos the problem of adaptation to mice occupied a special place in the study of influenza viruses. In connection with this, the method of neutralization with infection of mice lost its importance to a considerable degree.

Burnet suggested checking the degree of neutralization of the virus by serum by means of application of the mixture to the chorioallantoic membrane of the chick embryo. The virus-neutralizing properties of the serum thereby were determined by the absence of proliferative-necrotic foci of pathology in the chorioallantoic membrane (Beveridge and Burnet, 1946). Since the development of proliferative-necrotic foci is not characteristic for the influenza virus the recording of neutralization of the virus according to the presence of absence of foci may be applied only to individual, well adapted strains.

This method of serologic characterization of influenza viruses has not found practical application.

The Hemagglutination-Inhibition Reaction In Vitro and in the Egg. The serologic study of various influenza virus strains has become possible with the introduction of the hemagglutination reaction and of its inhibition by specific antiserum in vitro.

The hemagglutination-inhibition reaction has an advantage over the other neutralization reactions in the fact that it can be used immediately after isolation of the new

strain in embryos without any additional adaptation. Establishment of the existence of hemagglutination and determination of the hemagglutinin titer in the allantoic or amniotic fluid is an extremely simple method and can serve as an objective index for the calcuation of the dose of antigen in the reaction with the serum. The specific inhibition of the antiserum reaction is readily controlled, and nonspecific reactions are detected distinctly.

Hirst (1943, 1947) showed that the method of hemagglutination-inhibition reaction with immune ferret sera can be used to establish the similarity and difference of antigenic structure between influenza virus strains A and B.

In using the hemagglutination-inhibition reaction as a method of studying the antigenic structure of the influenza virus all the investigators have praised it highly. The hemagglutination-inhibition reaction has acquired specific importance in the works of comparative serologic study of a large number of strains. Chu, Andrewes and Gladhill (1950), Hilleman and others (1950), Isaacs and Andrewes (1951), Bozzo (1952), Magill and Jotz (1952) have shown that the hemagglutination-inhibition reaction is the best method for separating strains into serologic groups.

An essential defect in the hemagglutination-inhibition reaction is the presence of nonspecific inhibitors in the sera of experimental animals which are usually used for immunization (rabbits, ferrets) as well as in certain human sera.

The effect of the serum inhibitor is manifested in an inhibition of the hemagglutination reaction by the virus hemagglutinin in a mixture with normal animal serum. A marked depression of the reaction was found when normal sera reacted with freshly isolated strains.

Hirst (1948) suggested the treatment of rabbit's serum with a sodium periodate ($NaIO_4$) solution with subsequent removal of the excess iodine with a glucose solution or solution of glycerin. McCrea (1946) tested the heating of the sera to a temperature bordering on coagulation of the protein.

The works of Burnet, McCrea and Anderson (1947) have served as the main method for eliminating inhibitors. The authors found an enzyme which destroys the virus receptors of the erythrocytes in a filtrate of the cholera vibrio. Based on the Hirst conception of the similarity of erythrocyte receptors and serum nonspecific inhibitors, Mulder (1948, 1950) suggested using a filtrate of the cholera vibrio for the purpose of destroying the serum inhibitors. Since that time, this method has been adopted in many laboratories. The method is based on the effect of the enzyme at a temperature of 37°. An equal volume of filtrate is

added to the serum being investigated, themixture is set in a thermostat for 24 hours, and then the excess enzyme is inactivated by heating to 56° for 30 minutes (Appleby and Stuart-Harris, 1950).

A simpler method of eliminating the nonspecific inhibitors found in immune rabbit sera has been worked out by workers in the Institute of Hygiene at Warsaw, Kozinski and Walkowska (1952). The method consists in heating a mixture of the virus and the serum being investigated at a temperature of 37° for three hours before the addition of erythrocytes so that the live virus destroys the nonspecific inhibitors.

Therefore, the hemagglutination-inhibition reaction has been successfully utilized by many research workers as a method of studying the antigenic structure of influenza viruses. The main defect of this method was the nonspecific inhibition phenomenon which accompanied the specific reaction.

Additional work on the elimination of the inhibition, however, reduces the value of the method by complicating the reaction with the introduction of foreign ingredients or additional physical factors in the reaction of the main reacting components -- the antigen and antiserum.

The hemagglutination reaction and the specific inhibition of it by immune serum has been successfully used for the neutralization experiment in vivo, specifically in the developing chick embryo.

Neutralization reaction in ovo is a convenient method of serologic study of viruses (Hirst, 1942).

In this modification the neutralization reaction is accomplished according to the same system as previously, except that the mixture of virus and antiserum is not introduced into mice intranasally but rather is inoculated into the allantoic cavity of embryos which are then subjected to additional incubation with the aim of demonstrating the neutralizing properties of the serum.

The specific neutralization is defined by the absence of a hemagglutination reaction in the allantoic fluid of the embryo after 47-72 hours of incubation. The demonstration of hemagglutination indicates a multiplication of the virus with the development of hemagglutinins specific for the given serologic type.

By this method a study has been made of all strains isolated in embryos and adapted to them.

Sugg (1949) established the existence of antigenic differences between two variants of the CAM virus by this method; hilleman and others (1950, 1952) considered this method confirmed by the results obtained in the hemagglu-

tination-inhibition reaction. Archetti (1952), comparing antigenic differences between variants of the PR8 (A) and 965 (A-prime) variants in the hemagglutination-inhibition and neutralization reactions showed that the former reveals the differences which are not found in the neutralization experiment in ovo.

At the same time, Hultin and McKee (1952) studied the possibility of reactivation of a neutral mixture after injection of it into the allantoic cavity of an embryo. The authors succeeded in showing that the virus, which is neutralized by an excess of serum in order to prevent the production of hemagglutinin, is found in a state in which it is attached to the cells of the chorioallantoic membrane. Certain conditions which contribute to the reactivation of the mixture in the developing embryo have been analyzed by Isaacs (1948).

Therefore, the neutralization reaction in ovo, like the neutralization reaction in mice described above, is in certain cases dependent on the different degree of pathogenicity of the virus for the developing embryo. The pathogenicity of the virus for the embryo, which depends on the adaptation of the strain to the embryonic organism, can apparently play a decisive part in the matter of reactivation of a neutral mixture owing to the special tropism of the virus for the susceptible tissues of the developing embryo.

In the experiments of Gorbunova (1953) the neutralization and hemagglutination in ovo reactions proved to be more suitable than the neutralization of the pathogenic properties in mice; however, this reaction also has its defects.

The difficulty was the system of recording the neutralization reaction. The usual method of recording by means of determining theneutralization index was not very suitable. As is well known, the neutralization index is the ratio of the virus titer in the presence of immune serum to the titer of this virus in a mixture with control (normal) serum. This index gives us an idea of the strength of the virus neutralization by homologous serum compared with heterologous sera.

The neutralization of the virus with homologous serum is usually expressed as a high neutralization index in connection with the fact that the virus titer in the mixture with this serum is very weak (neutralization of massive doses of virus), whereas there is a high titer of the virus in the control mixture, which is taken as unity.

In Table 24 one of the protocols of an experiment for neutralization of the hemagglutinating properties of the influenza virus in ovo is presented. This experiment

gives us an idea of the merits and defects of the method. One of the main defects of it is the impossibility of making use of concentrated virus suspensions.

Table 24

Experiment in Neutralization of Influenza Virus Type A and the Shklyaver Strain With Immune Sera to Various Heterologous Strains and Antiserum for the Homologous Strain

Serum	Dilutions of Shklyaver Virus					Virus titer in Presence of Serum	Neutralization Index
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
Shklyaver	0 4	0 4	0 4	0 4		> 10^{-6}	
Mak	0 4	0 4	0 4	0 4		> 10^{-6}	
A30	0 4	0 4	0 4	0 4		> 10^{-6}	
PR8	1 4	1 4	1 4	1 4		> 10^{-6}	1 950
A36	2 4	1 4	1 4	0 4		1:26 300	740
A5	0 4	0 4	4	0 4		> 10^{-6}	
A4	0 4	0 4	0 4	4		> 10^{-6}	
Smor	1 4	1 4	1 4	0 4		> 10^{-6}	1 950
Yaks	2 4	1 4	1 4	1 4		1:28 900	670
Lahcho	3 4	1 4	1 4	0 4		1:45 700	436
Kri	4 4	4 4	2 4	2 4		1: 3 020 000	6
Normal serum	4 4	4 4	4 4	2 4	1 4	1: 9 500 000	1

Key: The numerator represents the number of eggs in the allantoic fluid of which a positive hemagglutination reaction was found; the denominator, the total number of eggs infected in the experiments.

By making use of this method neutralization experiments may be performed in ovo only with optimum dilutions of the allantoic virus, 10^{-4}-10^{-7}. This is explained by the fact that an accumulation of hemagglutinins in the allantoic cavity is possible only through the introduction of very much diluted virus material. The introduction of concentrated virus into the extraembryonic cavity involves the autointerference phenomenon, that is, the inhibition of accumulation of virus in the culture.

This characteristic feature of cultivation of the influenza virus makes it possible to establish the infectious nature of the virus according to its final dilution, but limits the study of the neutralization activity of the serum on account of the impossibility of using massive doses of the virus in the experiments. A control titration

of the virus (Table 25) gives us an idea of the inacceptability of the work with a virus more concentrated than 10^{-4}.

Table 25

Control Titration of Shklyaver Virus in Chick Embryo for the Purpose of Determining Hemagglutinins in Ovo

Dilutions of Virus in Physiological Solution									
10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
1/4	1/4	2/4	4/4	4/4	4/4	2/4	1/4	0/4	0/4

The key is the same as for Table 24.

Despite the impossibility of working with massive doses of the virus for the purpose of calculating the neutralization index in closely related or homologous sera (for the given strain) it is sometimes possible to compute the neutralization index anyhow. This gives us specific figured material for the comparison of the heterologous group of strains with one another by their antisera. Mixtures which indicate complete neutralization of the virus in its optimum dilutions for multiplication in the allantoic cavity which have not been subjected to any further analysis represent a broad field for guessing the maximum concentration of virus which can be neutralized by a given serum. Under these conditions it is not only impossible to establish the real serum titer in the neutralization reaction in ovo but it is also impossible to show any differences between related though not identical strains. For example, the Shklyaver, Mak, A39, A3, A9 strains in a concentration of 10^{-4} neutralize the Shklyaver serum equally but it can not be established by this method whether or not they are neutralized just as equally in dilutions of 10^{-3}, 10^{-2}, 10^{-1} and in ovo, since the control titration of the virus in these dilutions gives distorted results.

Without knowing the true titer of each serum in this reaction use may be made of the data of the hemagglutination-inhibition reaction for the purpose of selecting serum dilutions in each experiment. It seems to us that this principle of introducing another method into the experiment is undesirable.

In testing the serum dilutions corresponding to eight or four antihemagglutinating units less distinct results are obtained, apparently, because of a notable decrease in the neutralization effect.

Still, the great value of this reaction can not be overlooked, because it opens up considerably broader possibilities for the study of the antigenic structure of viruses in the neutralization reaction in ovo than was possible in experiments on mice.

The use of the neutralization reaction in ovo is expedient in carrying out certain parallel experiments with the hemagglutination-inhibition reaction, where it is necessary to elucidate the similarity of the strains. As follows from what has been presented above, the conditions of performing the experiment complicate the study of the finer antigenic differences between strains by the method of hemagglutination neutralization in ovo.

The complement-fixation reaction has been studied completely by many Soviet and foreign scientists. Naturally, in vitro reactions attracted the attention of research workers for the purposes of solving a purely serological problem -- the differentiation of strains without depending on the reaction between the virus and the living intact macroorganism.

Soviet investigators have worked out the method of the complement-fixation reaction and the application of it to the study of the antigenic structure and diagnosis of the influenza virus (Belikov, 1940; Nartsissov, 1944, 1948).

Peterson (1943, 1948, 1949), Yeremeyev and Peterson (1948) successfully used the complement-fixation reaction in the modification proposed by Ioffe and Rozental' (1943) - prolonged fixation in the cold -- for the purpose of detecting negligible quantities of the influenza antigen in washings from the respiratory passages of experimentally infected animals and for the purpose of diagnosing virus influenza in people.

Smorodintsev (1952) introduced a new system for performing the complement-fixation reaction in the cold, increasing the sensitivity of the reaction by means of increasing the volume of material examined for the presence of the virus antigen in the discharge from the pharynges of patients, in the blood and in autopsy material.

According to the reports of these and other investigators the complement-fixation reaction has been applied best to the determination of the increase in antibodies in the blood of those who have had influenza (Yurikas, 1950; Kaganskaya, 1952). The opinion has been created that the complement-fixation reaction is less sensitive in the differentiation between related strains of influenza virus

than the neutralization reaction.

Thus, Smith (1936), using a suspension of mouse lungs, which are readily infected by human and swine influenza viruses easily distinguishable in the neutralization reaction, as an antigen showed that the method of complement-fixation does not differentiate the antibodies to these strains. Lush and Burnet (1937), testing an antigen from the chorioallantoic membrane of the chick embryo, also came to the conclusion that the complement-fixation reaction is less specific than the neutralization reaction for the purpose of distinguishing the antigenic characteristics of two related strains of influenza virus.

Kaganskaya (1952) concluded that it was possible to demonstrate antigenic differences between strains of influenza virus by means of the complement-fixation reaction just as well as with the neutralization reactions and the hemagglutination-inhibition reaction. This conclusion was drawn on the basis of the fact that the use of a freshly isolated A-prime strain as an antigen in the complement-fixation reaction with the aim of diagnosing influenza by paired sera gave a higher percentage of positive results than the performance of experiments with the PR8 with the antigen. From our point of view this conclusion can not serve as an evaluation of the method of complement fixation for studying the antigenic differences between influenza virus strains but rather only indicate the expediency of using the antigen prepared from a strain which corresponds to the serologic type of virus which caused the outbreak.

Salk (1951) points out that the complement-fixation reaction is better suited to the demonstration of group antigens of the influenza viruses, and Ananthanarayan (1954) directs attention to the possibility of occurrence of the zone phenomenon in the performance of this reaction.

Gorbunova and Gavrilov (1954) made special investigations which had the aim of clarifying the suitability of the complement-fixation reaction method for the study of antigenic structure of influenza viruses. Since this reaction is frequently used in investigations, it seems expedient to discuss the results obtained in greater detail.

Allantoic cultures of influenza viruses constituted an ingredient of the reaction; rat immune sera were used (for the purpose of eliminating anticomplementary properties they were treated with carbon dioxide), the reactions were performed both in the usual manner and by means of fixation in the cold. For the purpose of a comparative quantitative record of the intensity of the crossed reactions the "intensity index" of fixation was used -- the arithmetic mean of the figures expressing the number of

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pluses in each individual case with consideration also of complete hemolysis, which was designated by zero.

Table 26

Calculation of the Degree of Complement Fixation with Consideration of All Reactions from 0 to 4 Plus

Antigen	Sera to strain	Dose of complement		
		1.25	1.5	2
PR8	PR8	44(4)	3344(3,2)	123(2)
Shklyaver	PR8	23(2,5)	2222(2)	0
A–prime	PR8	33(3)	1010(0,5)	0
B (Kri)	PR8	10(0,5)	1100(0,5)	0
PR8	Shklyaver	Not checked	232(2,3)	0
Shklyaver	Shklyaver		443(3,6)	222(2)
A–prime	Shklyaver		210(1)	0
B (Kri)	Shklyaver		100(0,3)	0
PR8	A–prime	1221(1,5)	1020(0,7)	0
Shklyaver	A–prime	1220(1,2)	1010(0,5)	0
A–prime	A–prime	3244(3,2)	3234(3)	222(2)
B (Kri)	A–prime	1111(1)	10(0,25)	0
PR8	Kri	22(2)	201(0,7)	0
Shklyaver	Kri	12(1,5)	0111(0,7)	0
A–prime	Kri	22(2)	2110(1)	0
B (Kri)	Kri	44(4)	4343(3,5)	2323(2,5)

In Table 26 the results are presented of several crossed experiments of complement-fixation between four antigens and their antisera (the intensity index of complement fixation is given in parentheses).

In evaluating the data presented in Table 26 it is easy to note, first, that the intensity index for each homologous reaction is greatest when compared with the indices of heterologous reactions under conditions of performing the experiment with the same dose of complement; secondly, the intensity index inevitably decreases if we compare the same cross reaction performed with three increasing doses of complement; third, under conditions of performing the experiment with two doses of complement the intensity index is shown only for homologous reactions, becoming zero in all the heterologous fixations. In Fig. 65, the comparative values of the intensity indices of the complement-fixation reaction are shown. The maximum intensity index is equal to four, that is, a complement fixation of four plus. Correspondingly smaller numbers express a reduction in the intensity of the reaction, which is very character-

istic of the manner of establishing similarities and differences between the strains.

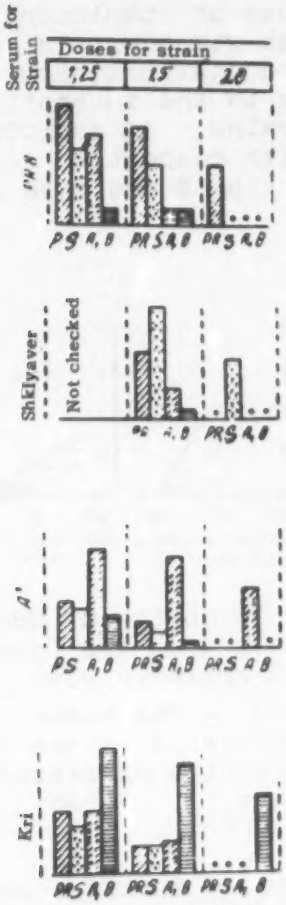

Fig. 65. Calculation of Intensity Indices of Complement-Fixation Considering All Reactions from 0 to 4 Plus. ["P" or "PR" means "PR8"; "Sh." means "Shklyaver"]

The comparative values of the intensity indices of the complement-fixation reaction are represented by the columns (Gorbunova and Gavrilov, 1954).

For the purpose of graphic demonstration curves are presented in Fig. 66 which give us an idea of the dynamics of the intensity of the reaction depending on the increase

in the dose of complement. Each graph represents the cross reactions of one serum with four antigens. In all the four graphs a definite tendency is shown toward a strict separation of the homologous reaction from all the heterologous reactions when the dose of complement is increased, although the intensity of these reactions is reduced thereby. In all the cases a marked distinction between strains A and B is observed according to the intensity index in the reactions between the strains. In addition, a distinction of the A-prime virus (with respect to A-prime serum) is seen both with respect to type B and both strains of A virus.

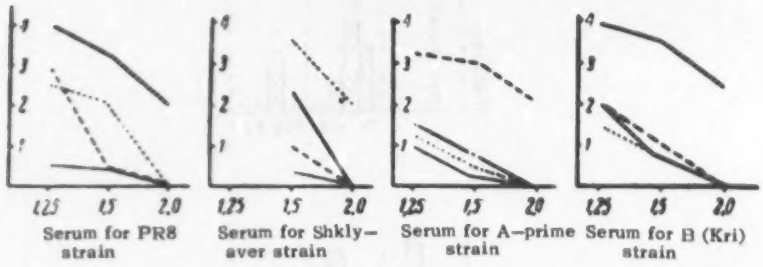

Fig. 66. Dynamics of Intensity of Complement-Fixation Reaction Depending on the Dose of Complement (Gorbunova and Gavrilov, 1954).

Along the abscissa axis -- the doses of complement (in units); along the ordinate axis -- the intensity index of the complement-fixation reaction expressed according to the number of pluses; antigens: PR8; Shklyaver; A-prime; (Kri).

At the same time, a definite relationship is observed between both A strains (PR8 and Shklyaver) which is expressed in a similarity of the intensity indices with respect to PR8 and Shklyaver sera with a dose of 1.5 units of complement.

Finally, in Fig. 67 the ratio of the intensity indices of the heterologous to the homologous reactions is presented. This calculation is based on the fact that each homologous index for the given serum is taken as unity with respect to the heterologous index within limits of the same dose of complement. Therefore, each hatched rectangle, consisting of 10 small squares is an entire antigenic unit. The number of hatched squares is proportional to the relationship of the antigenic structures of the individual strains.

The completely hatched rectangles are typical of the homologous reactions of PR8, Shklyaver, A-prime and B sera, regardless of whether the experiment was performed with 1.25; 1.5 or 2 doses of complement.

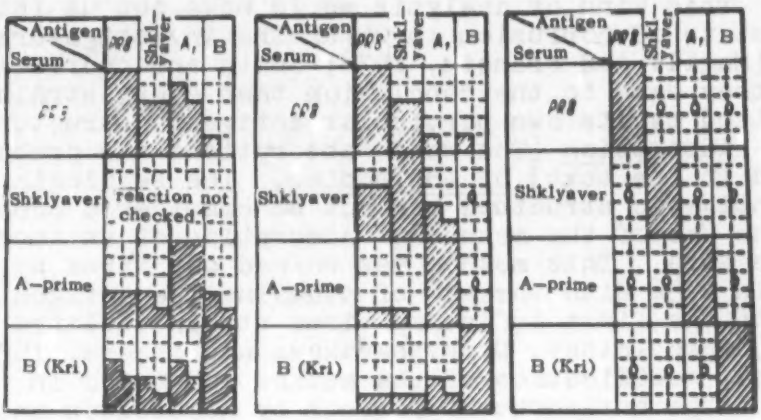

Fig. 67. Ratio of Intensity Indices of Heterologous to Homologous Complement-Fixation Reactions.

The rectangle made of 10 squares is an entire antigenic unit; the number of hatched squares is proportional to the degree of relatedness of the antigenic structure (Gorbunova and Gavrilov, 1954).

An analysis of the material presented leads to the conclusion that the results of the complement-fixation reaction which depend on the number of doses of complement used in the experiment are exceedingly labile. Actually, from Fig. 67 (1.25 units of complement) the conclusion may be drawn that the PR8, Shklyaver and A-prime strains are similar with respect to the PR8 strain antiserum and even that there is a slight kinship of them with the B strain (Kri). At the same time, the experiment using the dose of 1.5 units of complement makes it possible to establish firmly the close relationship between the A strain (PR8 and Shklyaver) and to relate the A-prime strains remotely in the same way, to the A strain in which these strains are related to the B strain. With a dose of complement of two units no relationships are found between the types of virus; thereby, only a specificity of the strains is found, that is, the independence of such serologic varieties as

the PR8 and Shklyaver strains is established within type A.

In general, it may be said that the complement-fixation reaction is of definite value for the purpose of classification of influenza virus strains. However, the absence of standard experimental conditions can lead to a subjective recording of the results, as seen from Fig. 67.

This kind of analysis would have put us into the same state of confusion in which the investigators were in 1938 (Magill and Francis, 1938; Smith and Andrewes, 1938) when they came to the conclusion that every strain is characterized by its own particular antigenic structure.

Adsorption [The word "absorption" was probably intended in the text] of Antibodies. The serologic study of the antigenic structure can not be considered complete without the use of the method of adsorption of antibodies from immune sera. This method was worked out first by Smorodintsev in 1936 with the aim of studying the antigenic structure of the first influenza virus strain isolated in the USSR (Smorodintsev, Drobyshevskaya and others, 1936). The original modification of the method consisted in the absorption of the antiserum of ferrets by homologous and heterologous virus strains in the form of mouse-lung suspension. The excess of virus in the mixture with the antiserum was removed by filtration through finely porous filters which did not allow the virus particles to pass through. The filtrate of the absorbed serum was again saturated with a virus suspension of the homologous strain. The completeness of adsorption of antibodies and the identical nature of the strains were established by the absence of a neutralizing effect of the serum on the homologous strain in the experiment of infecting mice.

At the present time, the method of antibody adsorption in its new modification, which consists of the adsorption of antihemagglutinins from immune serum, has been used successfully in Smorodintsev's laboratory for the study of the antigenic structure of influenza virus strains. Thus, Luzyanina (1951) studied the antigenic characteristics by the method of cross absorption of the sera by the corresponding strains.

Friedewald (1944) in experiments with the adsorption [should be "absorption"] of antibodies (antihemagglutinins) was able to demonstrate a high degree of specificity of the reaction. By this method the advantage of the PR8 strain over several strains of type A studied was established because of the richness of its antigenic components. On the basis of his experiment the author recommended the obligatory introduction of this strain into the composition of influenza vaccine.

Sigel (1949) utilized the method of antibody absorp-

tion, using sera absorbed by heterologous strains in the complement-fixation reaction. By this method the author established the existence of differences between the three B virus strains isolated during the same localized outbreak.

Certain authors, however, obtained negative results in studying the antigenic differences between the strains by the method of influenza antiserum absorption. Thus, Stuart-Harris, Andrewes and Smith (1938), using the method of absorption with subsequent testing of the virus-neutralizing activity of the serum in a mixture with the virus in experiments on mice drew the conclusion that this method did not contribute much to solving the problem of the antigenic structure of the strains.

Burnet and Lush (1938) were not successful in absorption experiments with the subsequent infection of mice but obtained better results in the neutralization reaction with absorbed sera with subsequent cultivation of the virus on the chorioallantoic membrane of the chick embryo.

Fulton and Dumbell (1949) considered the application of the antibody absorption technique of doubtful value for the study of the fine antigenic structure of influenza virus strains.

The efficacy of the method of antibody absorption for the study of the antigenic structure of influenza viruses was checked by Gorbunova (1953).

Allantoic fluids which contained the virus and which were used in the hemagglutination-inhibition reaction served as the antigens. In certain cases, the allantoic antigen was concentrated so that antigens with the same hemagglutinating activity participated in the experiment of absorption of antibodies from the same serum. The concentration was accomplished on chick erythrocytes in working with viruses of the influenza B type. The process of adsorption and elution of the virus was conducted in accordance with the description of this procedure by Sokolov (1949). A thick suspension of undiluted erythrocytes in excess was added to a definite volume of allantoic fluid with an inadequate hemagglutinin titer (less than 1:160). The mixture was left in the refrigerator for the night. In the morning the supernatant fluid was removed, the erythrocyte sediment with the adsorbed virus was suspended in 1/10 volume of physiological solution compared with the original quantity of allantoic fluid. The suspension was transferred to a thermostat for 30 minutes with repeated shaking every 10 minutes for the purpose of eluting the virus from the surface of the erythrocytes. After this, the mixture was centrifuged in beakers containing ice for the purpose of separating the erythrocyte sediment from the eluate. The freshly prepared eluate was titrated in the hemagglu-

tination reaction and used on the same day in an experiment of antihemagglutination absorption in the serum under study.

Therefore, a number of antigens was selected with the same hemagglutinin titer with respect to a single erythrocyte species. Antigens having a titer of less than 1:640 were usually not used. For the purpose of equalizing the titers the antigens were not only concentrated but in a number of cases they were also diluted with physiological solution up to the titer indicated.

The experiments of antihemagglutinin absorption were carried out only with the sera of rats which had been immunized, as mentioned above, by allantoic cultures of the influenza virus. The absorption of immune sera was accomplished according to the Smorodintsev method, which has been described in the work of Luzyanina (1951). The serum was used in a dilution of 1:16. Four cubic centimeters of the antigen were added to five cubic centimeters of the diluted serum. As many of these mixtures were made in separate test-tubes as there were antigens used in the experiment. The presence of completeness of absorption was established by the hemagglutination reaction, that is, by an excess of the virus in the mixture. Test-tubes containing a mixture of serum and antigens were left in the refrigerator for 24 hours, after which the undiluted erythrocytes were added in a quantity of one cubic centimeter for the adsorption of the virus excess at room temperature for 30 minutes. When the experiment was performed in this way the original serum dilution of 1:16 was doubled.

After centrifugation or complete sedimentation of the erythrocytes a second test was made for absence of the free virus in the serum being investigated, and only after this was the hemagglutination-inhibition reaction performed with the homologous virus strain.

The recording of the reaction was accomplished by comparing the titer in serum absorbed by heterologous strains with the titer of the same serum against the homologous strain. The serum titer against the homologous strain was determined by three control experiments. A titration was carried out with the homologous strain: 1) of serum absorbed by the homologous strain; 2) of serum which was in a mixture with normal allantoic fluid or with physiological solution under conditions of the absorption procedure; 3) of untreated serum, that is, serum which had not been subjected to any manipulations.

In the experiments of antihemagglutinin absorption from A, A-prime and B sera with various strains of A, A-prime and B viruses it was possible to obtain convincing results showing differences between the types and strains among the influenza viruses.

By means of the antihemagglutinin absorption reaction, just as with the hemagglutination-inhibition reaction, it was possible to establish qualitative and quantitative differences between the antigenic structures of various strains.

While it is to a certain degree a mirror image of the hemagglutination-inhibition reaction the antihemagglutinin-absorption reaction is a painstaking one and requires a greater expenditure of technical equipment, because a lack of agreement of the results is noted in repeated experiments. In certain cases an inactivation of the diluted serum is observed in the course of the absorption procedure (decrease of the titer by four to eight times).

In conclusion, it should be noted that the antihemagglutinin-absorption reaction is more complex than the hemagglutination-inhibition reaction. It is particularly difficult to observe uniform experimental conditions in working with sera of different serological activities and antigens with different hemagglutinating activity. As a result of this, the reaction is applicable in the differentiation of a small number of strains. As seen from Table 27, by means of the reaction of absorption of antihemagglutinins from sera it is readily possible to establish the identity of the strains, as is seen through the example of the PR8 and A36, Shklyaver and A39, Klim and Pan strains. However, it is not so easy to detect differences between closely related strains, and sometimes it is altogether impossible on account of the fact that a serum dilution of 1:16 is required. Smaller dilutions are not very acceptable because of the reduction in the specificity of the reaction observed.

Of the other methods of investigating the antigenic structure mention should be made of the precipitation reaction with purified virus which has been proposed by Knight (1946). Through the use of this reaction it was shown that the PR8 and Lee viruses grown on chick embryos contain, in addition to their own antigen, an antigen which is part of the normal allantoic fluid. Similar data have been obtained also with viruses cultivated in mouse lungs.

In addition, cross resistance experiments may be used in mice for evaluating the immunologic properties of various strains. Naturally, these experiments are possible only with strains which are pathogenic for mice.

Evaluation of Different Methods of Studying the Antigenic Structure of Influenza Viruses. In the literature there is little information on the comparative study of the sensitivity of the complement-fixation and hemagglutination inhibition reaction for the purpose of determining the various influenza virus strains.

Table 27

Hemagglutination-Inhibition Reaction in Experiments with Absorbed PR8 and Pan Sera

Serum	Absorption by strain	Dilutions of absorbed serum in the hemagglutination inhibition reaction with the homologous virus strain										
		1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
PR8	PR8	-	-	-	-	+++	+++	+++	+++	+++	+++	+++
	A36		-	-	-	+++	+++	+++	+++	+++	+++	+++
	Shklyaver		-	-	-	+++	+++	+++	+++	+++	+++	+++
	A39		-	-	-	-	+++	+++	+++	+++	+++	+++
	Klim (A–prime)		-	-	-	-	-	-	-	+++	+++	+++
	Pan (A–prime)		-	-	-	-	-	-	-	+++	+++	+++
	Kri (B)		-	-	-	-	-	-	-	-	+++	+++
	Physiological solution		-	-	-	-	-	-	-	-	+++	+++
	Normal allantoic fluid		-	-	-	-	-	-	-	-	+++	+++
	—		-	-	-	-	-	-	-	-	-	+++
Pan	Pan		---	---	+++	+++	+++	+++	+++	+++	+++	+++
	Klim (A–prime)		---	---	+++	+++	+++	+++	+++	+++	+++	+++
	PR8		-	-	-	-	+++	+++	+++	+++	+++	+++
	A36		-	-	-	-	+++	+++	+++	+++	+++	+++
	Shklyaver		-	-	+++	+++	+++	+++	+++	+++	+++	+++
	A39		-	-	+++	+++	+++	+++	+++	+++	+++	+++
	Kri		-	-	-	-	-	+++	+++	+++	+++	+++
	Physiological solution		-	-	-	-	-	+++	+++	+++	+++	+++
	Normal allantoic fluid		-	-	-	-	-	+++	+++	+++	+++	+++
	—	-	-	-	-	-	-	+++	+++	+++	+++	+++

Key. /// Absence of hemagglutination-inhibition reaction; -- hemagglutination-inhibition reaction.

Nartsissov and Parnes (1948) studied the complement-fixation reaction and the hemagglutination-inhibition reaction as applied to the serum diagnosis of influenza with paired sera in comparative experiments. The authors obtained a complete correlation in the results obtained.

Smorodintsev (1951), based on considerable experience in the diagnosis of influenza, points out that by the complement-fixation reaction it is possible to determine

the increase of antibodies in the convalescent sera in 85 percent of the cases, whereas it is possible to do this by the hemagglutination-inhibition method in only 51 percent.

The advantage of the complement-fixation reaction, in the author's opinion, consists of the fact that the results are not dependent on the characteristics of the intratype antigenic structure of the A or B antigens used. In contrast to this, the results of the hemagglutination-inhibition reaction depend on the specificity of the given strain. On the one hand, it seems to us, this speaks for a higher degree of sensitivity of the complement-fixation reaction than the hemagglutination method, but, on the other hand, it also speaks for the impossibility of distinguishing the antigenic variants not only of related strains but also of such remote strains as A and A-prime by the method of complement fixation in these experiments. Hence, it is understandable that the complement-fixation reaction is not a method for the fine differentiation of strains.

The work of Fulton and Dumbell mentioned above is interesting from the point of view of an evaluation of the methods being compared and the results obtained. The authors see the chief advantage of the hemagglutination-inhibition reaction over the others in the simplicity of its technique. They, like Hirst, consider the absence of a standard method of making the various series of erythrocytes and the unequal avidity of the antigen for combining specifically with the antiserum defects in the reaction. The main difficulty in application of the hemagglutination-inhibition reaction is reduced by these authors to a nonspecific inhibition of fresh strains by normal sera, which has been described by Francis and others. Although the complement-fixation reaction is more difficult to perform, it, in the authors' opinion, gives more distinct results. For purposes of simplification of the method the author suggested the droplet method on a plate with cups for the purpose of performing the complement-fixation reaction.

The work had the aim of comparing the antigenic interrelationships between seven strains which had been well studied by other authors using the method of the hemagglutination-inhibition reaction.

Fulton and Dumbell, taking into consideration the results of the complement-fixation reaction, established the antigenic characteristics of the strains, on the basis of which a conclusion may be drawn only that there is a considerable difference between the group of strains of type A, including A-prime, and the group consisting of the two B strains. We should like to emphasize particularly that in these experiments the authors did not succeed in

differentiating the A-prime from A strains.

It should be noted that the authors did not perform any comparative experiments by the method of hemagglutination inhibition, but the more distinct differences between the A (PR8, WS) and A and A-prime (Rhodes, Bar) strains in the hemagglutination-inhibition experiments of Francis and Quilligan (1947) and Dudgeon (1948) are well known.

Salk (1951) made a more critical approach to comparing the methods of complement fixation and hemagglutination inhibition. Considering that the hemagglutination-inhibition reaction has the capacity of detecting the most insignificant antigenic differences between strains and that the complement-fixation reaction can detect the more general related qualities, he made it his problem to show these properties experimentally. In artificially prepared mixtures of equal quantities of virus A and B (PR8 and Lee) he attempted, but unsuccessfully, to identify the strains by the method of hemagglutination inhibition, but he showed both components in the mixture by the method of complement fixation.

On this basis, Salk denies the sensitivity of the hemagglutination-inhibition reaction. When put in this way, the problem, in our opinion, does not constitute an answer, in essence, to the capacity for distinguishing fine antigenic differences between the various strains. In Salk's experiment the property of serum for reacting specifically only with the Pr8 strain was shown in the hemagglutination-inhibition reaction when the hemagglutinating activity of the Lee strain was maintained, and vice versa. Naturally, in the complement-fixation reaction the fixation is demonstrated, despite the presence of an unfixed component in the nixture.

The author's suggestions that this kind of serum effect occurs on the mixture of A and A-prime viruses have no basis, because no cross experiment was performed between A and A-prime viruses. At the same time, it is incomprehensible why not a single virus (A or B) was found in the mixture by the hemagglutination-inhibition method if the author knew the original titers of both sera.

Isaacs and Andrewes (1951), after the study of the antigenic structure of 70 strains, indicate the fact that the serologic differences found as a result of the hemagglutination-inhibition reaction are not revealed by the method of complement-fixation. Chu, Andrewes and Gledhill (1950) report on the lesser degree of suitability of the complement-fixation reaction for studying the antigenic structure of the strains.

Therefore, an analysis of the data in the literature concerning the selection of the best method for studying

the antigenic structure of the influenza virus leads to contradictory conclusions. However, a certain conclusion may be drawn on the basis of what has been stated. It should be pointed out that the method of antiserum neutralization of a virus pathogenic for mice has lost its importance after the change-over to the technique of isolating strains in chick embryos.

Instead of neutralization of the infectious properties of the virus in experiments on mice the method of hemagglutinin neutralization was suggested in experiments on eggs. This method has become widespread and has merited a good appraisal in its application to the antigenic analysis of the strains, although there are indications of its lesser degree of sensitivity compared with the method of hemagglutination inhibition in vitro.

At the present time, two methods of serologic analysis of the strains in vitro have become most widespread -- the hemagglutination-inhibition reaction and the complement-fixation reaction. The former is exceptionally simple to perform; the latter presents a certain difficulty in connection with the complexity of performing the reaction. The comparative study of both methods has not been much discussed in the literature. All the investigators point out that the value of the hemagglutination-inhibition reaction is considerably lessened because of the nonspecific hemagglutination inhibition of freshly isolated strains by normal sera of laboratory animals.

The serologic study of influenza virus strains by the method of serum absorption has lost its importance. From the point of view of antigenic analysis of the virus-neutralizing antibodies, however, the method has acquired new importance in its application to antihemagglutinin absorption.

27. Development of Research on the Study of the Antigenic Structure of the Influenza Virus

The study of the antigenic structure of the influenza virus may be divided into three historical phases.

The first phase may be considered the period from 1933 to 1940, at which time the idea of the serological types of virus was fitted into the concept of an absence of complete identity of the strains related to one another.

The second phase was associated with the recognition of separate, serologically independent types of A and B viruses, that is, beginning with the time of discovery of the first type B virus strain (1940).

The third phase was the study of the serological variability of the influenza virus. The beginning of this

period should be considered the discovery of the A-prime viruses, that is, 1947. A complete study of the antigenic structure of the influenza virus from the point of view of variability has been undertaken at the present time in the laboratories of the Soviet Union and by many foreign investigators.

First Attempts at Separating the Influenza Virus Strains into Serological Types. In 1935, Francis and Magill, and in 1936, Sugg and Magill, indicated the possibility of the existence of related but not identical strains of influenza virus isolated from different people at different times.

Smith, Andrewes and Laidlaw (1935) in crossed immunization experiments in animals established the identity of two strains of human origin (WS-1933 and BW-1934) and the absence of identity of the swine influenza strain with the human strain. These investigators isolated a number of strains during the period 1933-1936 which were identical with the WS virus. On this basis, Stuart-Harris, Andrewes and Smith (1938) prepared a vaccine from the WS strain and immunized large groups of people in different regions of England before the start of an outbreak in 1936-1937. After noting the failure of their vaccination, the authors established the fact that the strains isolated during the outbreak were different from the vaccinal strains.

Studying the new and old English strains as well as the strains obtained from other countries in experiments of crossed neutralization with immune sera of ferrets, the authors established the existence of differences between the various strains. It was possible to distinguish three serologic groups of strains. These were group I: WS-BH (England), Sil (Leningrad); group II: PRS (American), Kor (Germany), Tal-Bav (England), Viz (Hungary); group III: Gat (England). Despite the difference in the strains in experiments with ferret sera they were shown to be very similar in neutralization experiments with hyperimmune horse serum. The authors believed that all the strains have a common antigenic factor and the differences are not so great as, for example, the differences between the three virus types of foot-and-mouth disease. It seemed very probable to the authors that somewhere a strain exists which coincides quite well with all the serologic types known at that time (master strain).

As the next problem in the matter of controlling influenza during this period many authors considered it most expedient to look for a strain among those newly isolated from people and among the previously known strains which might assure the success of vaccination because of its hav-

ing all the components which had been encountered in its antigenic structure. This idea of the existence of a hypothetical principal, basic master strain was for a long time predominant in the minds of foreign investigators, which may be judged by the statements of Friedewald (1944) and then of Blake (1948) with regard to the development of the problem of vaccinating people against influenza.

In 1938, Magill and Francis, on the one hand, and Smith and Andrewes, on the other, undertook an extensive investigation on the antigenic differences among influenza virus strains which had been isolated at that time by various authors in various countries.

Magill and Francis (1938) made use of the method of crossed neutralization of the virus, cultivated in surviving tissues in vitro, with serum obtained by means of immunization of rabbits. The results of the neutralization were checked by intranasal infection of mice with homologous and heterologous mixtures of 24 virus strains with antisera. In addition, experiments of cross immunization of mice were performed with 17 strains of tissue-cultured virus.

Smith and Andrewes (1938) studied 28 strains the majority of which had been included in the experiments of American investigators. The authors utilized three serological methods: 1) cross neu ralization in mice; 2) adsorption of neutralizing antibodies; 3) corss immunization of mice. In contrast to the experiments of Magill and Francis the English authors made use of the virus in the form of a filtrate of mouse lungs instead of tissue cultures and of a serum from covalescent ferrets instead of rabbit serum. In view of the fact that the filtrate of the mouse lung suspension was weakly virulent the neutralization and cross immunization experiments were not evaluated according to the principle of protection against a lethal pneumonia but rather on the basis of the prevention of pulmonary pathology in the experimental mice.

Magill and Francis as well as Smith and Andrewes established the difference between strains isolated during various epidemics and during the same epidemic. They point out that notable differences exist between certain strains and that there is a striking similarity between others. The authors noted the difficulty in evaluating the antigenic properties in cross neutralization and cross immunization experiments on mice, since the results frequently depended on the different degrees of virulence of the strains. In the works of both groups of investigators mention is made of the absence of reciprocity in the crossed reactions. In attempting to give a firm basis to the difficulty in the situation of work on antigenic analysis, Magill and Francis

drew the conclusion that the structure of the influenza virus is a mosaic of many antigens which are located in the depth and on the surface of the virus particle, so that practically every strain possesses its own antigenic structure.

Smith and Andrewes rejected cross neutralization of each of the 28 strains with each serum, because, in their own words, "the more strains included in the investigation the more difficult it was to detect any order in the chaos of results of cross neutralization." The authors limited themselves to experiments with sera prepared for the three strains which had been studied previously, WS, Talmeyn and Gateby, and a fourth, the Cpr strain, which in their opinion was specific. In these experiments it was noted that the American strains (PR8, Phila and others) belonged to an intermediate group between TAL and CHR and were closely related to one another. At the same time, Magill and Francis also saw the fundamental differences between the various American strains which had been established by Englishmen for English strains.

In summarizing the experiments of crossed neutralization, Smith and Andrewes pointed out that "the cause of the disagreement in results obtained on the two sides of the Atlantic Ocean remains unclear."

Experiments on the adsorption of neutralizing antibodies did not introduce any clarity into the attempt to come to unanimous conclusions as to the problem of the antigenic structure of the strains being analyzed.

It should be considered, therefore, that in 1938 it was impossible for the two groups of authoritative investigators to reach any agreement in the attempt to classify strains according to their serologic properties.

Burnet studied the antigenic structure of the influenza virus strains, making use of ferret antisera and the method of virus cultivation on the chorioallantoic membrane of chick embryos. The author found that the PR8 and Phila strains were closely related to one another and were different from the WS strain (Burnet and Lush, 1938).

In 1938, therefore, there were data in the literature indicating the difference between the PR8 and WS strains. However, the authors observing these differences did not ascribe any definite importance to them and did not consider it possible to formulate any kind of grouping of the other strains around these standard strains. Apparently, it was impossible in experiments on mice to establish distinctly any essential differences by the methods of crossed immunization, neutralization and antibody adsorption.

In the attempt to identify 18 strains isolated during

an outbreak of influenza in 1941, Gorbunova (1943) established the fact that only part of the strains were readily neutralized by influenza antiserum which had been prepared for the Leningrad strain of Smorodintsev. Evidently, during this epidemic strains of different serologic groups had been isolated.

Hirst (1943) investigated 18 strains of A virus, using the hemagglutination-inhibition reaction for the analysis of their antigenic structure. The strains which were isolated in 1940-1941 in various regions of the United States of America had a very similar antigenic structure. Two strains (WS and PR8) were notably different from them and from each other. The work was carried out with the aim of showing the suitability of the method used for the antigenic analysis of influenza viruses. At the same time, outbreaks and epidemics of clinically expressed influenza were described (Smorodintsev (1938), Francis and others (1937), Horsfall (1940)), in which it was impossible to isolate the virus in ferrets or mice or to find any increase in the antibody titer in the covalescent sera against the strains of influenza virus which had been known previously.

The Establishment of International Nomenclature for the Pathogens of Virus Influenza. In 1940, the need matured for creating an authoritative commision for the establishment of an international nomenclature of the influenza virus pathogens. Cases of the disease produced by any of the strains of virus similar to the Smith, Andrewes and Laidlaw strains were designated influenza A. In the event other viruses were isolated in diseases clinically similar to influenza A it was suggested that they be called viruses B, C, etc. (Horsfall, Lennette and others, 1940).

The discovery of type B viruses in the same year, 1940, confirmed the existence of a profound difference in the biological properties among the influenza virus pathogens. Thus, in addition to the marked serological individuality of the B strains other biological properties of the viruses of this type are known, for example, the difficulty in adaptation of the B virus to laboratory animals. This fact may be explained by the failure to isolate the virus in ferrets and mice in the outbreaks of clinical influenza described by Smorodintsev, Francis and Horsfall.

Lukashevich (1949) studied a passaged type B strain with respect to the increase in its virulence for mice. The author succeeded in obtaining a notable increase in the biological activity only after 20 serial passages.

Although with the transition to the technique of isolating strains in the amniotic cavity of chick embryos in 1945-1946 reports appeared concerning the isolation of

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type B viruses in various countries, influenza epidemics caused by the B virus were diagnosed only by the increase in antibody titer in covalescent sera with respect to standard type B strains.

The establishment of the new serologic type B, therefore, put the minds of research workers at rest, because the possibility had appeared of deciphering each outbreak and even every case of sporadic influenza as type A or type B influenza, if not on the basis of isolation of the strain them by means of serologic analysis of patients' sera with one of the standard diagnostics. Thus, by means of studying paired sera an outbreak was characterized as influenza B in the USSR in 1949 by Peterson and others (1949), Shimshilevich, Smolyanskaya and others (1949), Smorodintsev, Rappoport and others (1950), Rappoport (1950), Chalkina and others (1950), Solov'yev and Zakstel'skaya (1951), Berlyant and Orlova (1951), Neklyudova (1951) and Kornyushenko (1952).

The distinct antigenic difference between A and B viruses made it possible to work out methods of differential diagnosis, to detect the etiology of virus influenza and acute catarrhs of the upper respiratory tract. The problem of the immunology of influenza and the administration of prophylactic inoculations were studied and worked out in accordance with the recognition of the existence of two serological types of virus influenza, A and B. Being guided by the serological data on the existence of two types of influenza viruses, A and B, the Commission on Influenza in America undertook the vaccination of military contingents. The vaccination against an epidemic of type A was successful in 1943-1944, and in 1945 against a type B epidemic.

The Antigenic Structure of A and A-Prime Viruses. The standard American vaccine did not exert any effect during the epidemic of 1946-1947 in the United States of America. The cause of failure soon became clear, because the strains which had been isolated during this epidemic were shown to be not only not identical with the vaccine strains (PR8, WS, Lee), but even completely different from them in the antigenic respect.

The Rhodes strain isolated during this period by Francis, Salk and Quilling (1947) was tested with convalescent sera. It was shown that in convalescent sera a rise in antibodies was found both against the new Rhodes strain and the PR8 strain but not against the Lee strain. On the basis of this, it was established that atypical A virus strains were the cause of the epidemic. In the sera of convalescent ferrets which had been infected with the Rhodes strain a high titer of antibodies was found against this strain, but no antibodies were found against the old A, PR8

and WS strains.

Therefore, only a very remote similarity could be established between the new strain and the A viruses.

During this period, Rasmussen and others (1948) and Stokes and Smadel (1948) isolated four strains among those vaccinated with a standard vaccine at military installations located in another part of the American continent. These strains (FM, LF1, LF2 and SF1) were not very similar to the strains of previously known types in their antigenic structures either. Table 28, which has been taken from the work of Rasmussen, demonstrates these differences between the strains in the experiment of a crossed hemagglutination-inhibition reaction.

Table 28

Identification of 1947 Strains with Those Previously Known According to Rasmussen and Co-authors (1948)

Serum / Virus	FM1	LF1	PR8	Swine	Lee
FM1	1:2048	1:1025	1:16	0	1:16
LF1	1:1024	1:512	1:32	0	1:16
SF1	1:1024	1:128	1:64	—	1:16
PR8	1:64	0	1:4096	0	1:16
Weiss	1:16	1:8	1:512	0	—
Swine	1:32	1:16	1:64	1:1024	—
Lee	1:32	1:16	1:16	—	1:2048

Key. The figures indicate the serum titers (dilutions).

From Table 28 it is seen that the three new strains used in the experiment -- FM1, LF1 and SF1 -- comprise a separate group which is just as far removed from the typical PR8 (A) strain as the virus of swine influenza and Lee virus (B).

Specifically, PR8 sera with a titer of 1:4096 neutralize the FM1, LF1, SF1 and swine strains in the same low dilutions (1:16 - 1:64). Sera against the new strains do not exert any neutralizing effect on the PR8, Weiss, swine or Lee strains either.

Therefore, beginning with 1947 reports of a new serologic variant of the influenza virus began to appear in foreign literature. Afterwards, this variant obtained the

name "A-prime" (A').

With the isolation of the first A-prime virus strains in various countries the comparatively mild clinical course of the disease and the localized nature of the epidemic outbreaks were noted.

An analysis of the material in the literature showed that the A-prime strain (A128) apparently was first isolated in Sweden by Löfström (1945, 1949) during a small sporadic outbreak of influenza in 1944 but was first identified by the author only in 1949 after the second epidemic of 1947 had spread through the entire country. During this epidemic the author isolated a strain which proved to be similar to both the A128 (1944) strain and the standard American A-prime -- FM1.

The next date of an A-prime virus isolation refers to 1945-1946 when Anderson and Burnet (1947) obtained the CAM strain from a localized outbreak in Victoria (Australia).

Identification of the CAM strain was accomplished later by Rasmussen who found a considerable similarity between this strain and the FM1 strain which he had isolated.

In England, Dudgeon and others (1948) observed localized outbreaks of the influenza type from January through March 1947. The authors isolated about 30 strains of virus which afterwards were identified as A-prime. Of this group of viruses the Bar (Barret) strain was afterwards used by certain research workers as a typing A-prime strain. A number of strains was isolated in subsequent years in England.

In March 1947 numerous virus strains of A-prime influenza were isolated by various authors in America, and of these the FM1 strain was considered the standard for identification of them.

Taylor (1949) isolated a number of strains in New York and its suburbs, the majority of which were similar to the FM1 strain.

Ward and Eddy (1950) isolated about 30 strains of the A-prime virus in the area of Baltimore, and Siegel (1949), five L47 strains similar to the FM1 and CAM strains.

In 1948-1951, American investigators continued to report the isolation of all the new strains having an antigenic similarity to the FM1 strain. Thus, Archetti and Horsfall (1950) isolated the SH strain in New York; Hilleman, Mason, and Rogers (1950), the PW1-50, FM2-50, AH1-50, AD1-50 strains in Georgia and Wyoming; Campy and Weiss (according to Hilleman), the Wilfong, SF1-49 strains in California; Dempster (1952), 10 strains in Canada.

In Western Europe in 1947-1949, as has been mentioned above, the G-g strain was isolated by Löfström. Mulder and co-workers (1949) isolated a series of strains

in Holland; workers in the Pasteur Institute (Cateigne and others, 1949) in Paris isolated the D48 strain.

From the data presented it is clear that epidemic outbreaks of virus influenza in which A-prime stains were isolated have been noted in America, Australia, England and Western Europe beginning with 1945-1946.

In 1951, the World Center for the Study of Influenza in London undertook the study of strains isolated in 1950-1951 in the countries of the Eastern Hemisphere. The serologic study of about 90 strains made by Isaacs and Andrewes (1951) showed that they all belonged to the A-prime type. A detailed analysis of their properties made it possible to divide these strains into two subtypes which were designated the Scandinavian and Liverpool types by the authors (see below).

There is no information on the isolation of A-prime strains in the USSR in the period between 1946 and 1948 inclusive.

Chalkina and Zak (1950) in the laboratory of A. A. Smorodintsev in January-February 1946 isolated 16 virus A strains.

The new strains were homogeneous with respect to their antigenic structure and were different from the laboratory A strain only in their lower degree of pathogenicity for white mice. One of these strains (A39) was transmitted among the group in our laboratory in June 1951, and we classified it in the group of A strains which are similar to the WS virus in their antigenic structure. At the beginning of 1946, during an outbreak of influenza in Moscow, Khayt and Gorbunova (1947) isolated 18 strains of influenza A virus in mice. Evidently, these were typical strains, because their identification and the accumulation of antibodies in the sera of convalescents offered no difficulties during the work with diagnostic preparations prepared from the laboratory strains of A virus.

Fal'kovich (1949) isolated the MS12 strain in Moscow in 1948 which she identified as an A virus belonging to the PR8 group.

In December 1948, Gorbunova (1950) isolated three strains of influenza A virus (Mak, Shklyaver, Sher) similar to the antigenic WS group. It was only through the epidemic outbreaks of virus influenza in the USSR in March-April 1949 that material was obtained for isolation of atypical strains of viruses which afterwards were identified as A-prime in various cities of the Soviet Union.

Gorbunova (1951) in Moscow isolated 20 strains of virus (S3, S6, Sdb, Lshcho, Yaks, Gavr, Bel, YeM, Chizh, Ku, Klim and others). First, the biological properties of 11 of these strains were studied from the point of view of

O-D variability (see below); then, at the Third Scientific Session of the Institute of Virology of the Academy of Medical Sciences USSR 30 January-1 February 1951 Gorbunova (1950, 1951) gave a report on their antigenic characteristics. On the basis of cross-hemagglutination reactions with corresponding antisera it was established that the group of type A viruses can be divided into the following three categories: 1) strains similar to WS, 2) strains similar to PR8, and 3) a distinct group of strains isolated in March 1949 which are markedly different from the first two groups of A viruses. In 1950, one of the new strains (YeM) was identified as a type A-prime strain by means of comparison with a standard FM1 strain (Solov'yev, 1951).

At the same session of the Academy of Medical Sciences USSR Romanenko gave a report (Romanenko and others, 1950) on the strains of influenza viruses which he had isolated in Leningrad in March-April 1949 which were essentially different from the laboratory PR8 strain.

Smorodintsev and others (Smorodintsev, Vlastelitsa and others, 1950) reported on the virus strains which they had isolated during an increase in the number of cases of influenza in Leningrad in March-April 1949. A study of 16 isolated strains by various serologic and immunologic methods showed that only one of them, namely, the 1949-4 strain, was very similar to the laboratory PR8 strain. The great mass of other strains of the 1949 vintage showed marked antigenic and immunogenic differences from the A strains previously encountered in the USSR (Luzyanina, 1952; Morozenko and Vlastelitsa, 1953).

In 1951, a report appeared from the laboratory of Khodukin (Maksianovich and Il'yina, 1951) in Tashkent concerning the isolation of an atypical influenza virus from a patient with influenza in the spring of 1949. The authors came to the conclusion that the Tl-49 strain, which is different from the A and B viruses, is a new type virus. Testing of the Tl-49 strain with antisera from atypical strains isolated by Romanenko in 1949 showed the identity of the Tashkent and Leningrad strains in the hemagglutination reaction.

In subsequent years many A-prime strains were isolated in the USSR.

We should like to note certain works in which these strains were studied in detail. Ritova (1952), during an outbreak of influenza in the winter of 1952, isolated 11 strains which were all identified as A virus strains. In the same year, similar strains were isolated and studied by Solov'yev and Gutman (1952). Luzyanin (1953) isolated 17 strains of which only three were similar to the A viruses previously isolated; 12 were similar to the A-prime

viruses, and two strains were poorly neutralized by the A and A-prime sera (Fig. 68). In 1953, Ritova (1954) again isolated a number of strains which belonged to the group of A viruses and which varied in their antigenic structure (Fig. 69).

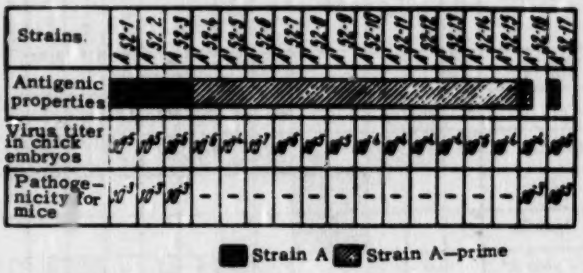

Fig. 68. Characteristics of Influenza A and A-prime Strains Isolated in 1952 (Luzyanina, 1953).

Therefore, in the years following the discovery of the influenza A viruses a considerable variety has been noted in the antigenic structure of the strains isolated belonging to this group of viruses.

Antigenic Structure of B Viruses. After the isolation of the first two strains in 1940, Lee by Francis (1940) and TM by Magill (1940), reports appeared concerning the epidemics produced by a new serologic type of virus similar to the Lee strain in its antigenic structure.

Thus, in 1941 Eaton and Bek in California isolated the Montgomery strain. In 1942, Nigg and others isolated two strains from material which had been preserved since 1939. Gordon (1942) isolated the No 572 strain from material taken during the epidemic of 1940 on the Island of Cuba. Taylor and others, according to the data of Burnet, Beveridge and Bull (1944), isolated the Argentine strain in 1941.

The identification of these strains offered no difficulties, since they were more or less similar to the Lee and TM strains in their antigenic and biologic properties.

In 1945 an epidemic of influenza B was noted in the United States, at which time a large number of strains was isolated. The new strains proved to be distinct from the Lee prototype strain. Many authors ascribed serologic differences found between the B strains of the 1940-1942 vintage and the B strains of 1945 to the different degree of adaptation of them to the host organism, since the former had been isolated in ferrets or hamsters, and the latter, in chick embryos. Among the latter, the Czech, Chaddick and other strains served as the standard ones in the study

of the antigenic structure of the B strains.

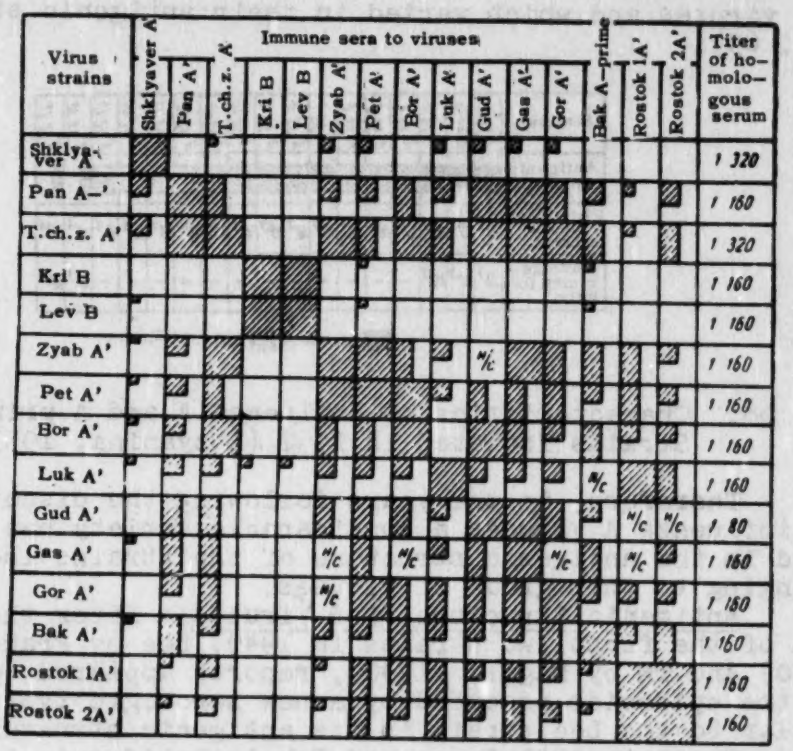

Fig. 69. Antigenic Characteristics of Influenza A-prime Virus Strains Isolated in 1953 (Ritova, 1954). The magnitude of the hatched area is proportional to the degree of antigenic similarity; n/p -- experiment not performed.

In Burnet's laboratory in Australia B strains were isolated which were not identical with the Lee strains: in 1944, the Bon strain (Burnet, Beveridge and Bull, 1944); in 1948, the Warner strain (Hilleman, Mason and Buscher, 1950).

During the epidemic of 1945-1946 several B strains were isolated which were similar to the Australian strains but not to the Lee strain. The Crawley strain, isolated by Dugeon and others (1946) belongs to this group of strains.

In 1946-1949-1950-1951 a number of B strains was isolated in different countries; the majority of them were related to the 1945 strain, and only a part of them were related to the Lee strain.

In the USSR, in Moscow and Leningrad, during the December outbreak in 1949 the Kri, S-8, Laz, Ts, B49 and other strains were isolated which differed considerably from the Lee strain (Gorbunova, 1952, 1953; Klyachko, 1953) and from previously isolated B virus strains (Rappoport, Gulamov and Chalkina, 1953). In December 1954, strains of B virus were isolated in Moscow which differed markedly from the Lee strain (we shall present their serological characterization below).

In general, the entire problem of the antigenic structure of theB viruses proved to be less complicated than that of the A and A-prime viruses.

28. Serologic Classification of Influenza Viruses

The complexity of and confusion in the problem of the antigenic structure of the influenza viruses and the absence of any sound classification of them stimulated us (Zhdanov and Gorbunova) to occupy ourselves particularly with the development of a classification of influenza viruses based on the study of their serologic properties (or, correspondingly, of their antigenic structure). These investigations, begun by Gorbunova in 1948, were for the most part, completed in 1952, at which time the classification of influenza viruses which we proposed was published (Zhdanov and Solov'yev, 1952; Gorbunova, 1952). Afterwards, the proposed classification was published in a number of works (Zhdanov, 1952, 1953, 1954; Gorbunova, 1953).

The first stage of the investigations was the division of the A viruses into two serological groups, WS and PR8. The separation of these two serologic varieties of A virus was based on the use of various methods of investigation. The most distinct results were obtained through the use of hemagglutination-inhibition reaction with rat sera (Table 29), which made it possible to divide the virus strains isolated up to 1948 inclusive into the two serological groups mentioned.

The new strains of A virus isolated in December 1948 (Mak, Shlyaver) could be categorized as one of the varieties of the A virus, namely, in the WS group (Table 30).

Afterwards, these strains were selected as standard strains in carrying out investigations of the antigenic structure by other methods. The correctness of dividing the A virus strains into two serologic groups was confirmed also through the use of other methods of investigation --

in experiments of cross absorption of antihemagglutinins (Table 31), in experiments of cross neutralization in eggs (Table 32), in experiments of virus neutralization in mice (Table 33) and in the performance of the complement-fixation reaction.

Table 29

Cross Hemagglutination-Inhibition Reaction of Various A Strains with Rat Immune Sera

Rat Sera	Hemagglutination Inhibition Reaction Titers With Strains					
	PR8	WS	№ 3	№ 7	№ 14	MS 12
PRS	1:320	1:20	1:40	1:20	1:20	1:320
WS	1:10	1:160	1:160	1:320	1:160	1:20
№ 3	1:20	1:160	1:160	1:160	1:160	1:20
№ 7	1:20	1:160	1:80	1:80	1:80	1:20
№ 14	1:40	1:160	1:320	1:160	1:320	1:40
MS 12	1:320	1:20	1:20	1:20	1:20	1:320

Table 30

Similarity of the Mak and Shklyaver Strains After 11-12 Egg Passages with the English WS strain in Accordance with the Result of the Cross Hemagglutination-Inhibition Reaction

Sera	Serum Titer of Hemagglutination–Inhibition Reaction With Strains			
	PR8	WS	Mak	Shklyaver
PR8	1:2 560	1:320	1:320	1:320
WS	1:320	1:2 560	1:1 280	1:1 280
Mak	1:320	1:1 280	1:2 560	1:2 560
Shklyaver	1:320	1:2 560	1:2 560	1:2 560

Table 31

Antigenic Relationships Between A Virus Strains (PR8 and Shklyaver) in Experiment of Cross Antihemagglutinin Depletion

Serum		Virus (four hemagglutinating units)	Serum dilutions in hemagglutination-inhibition reactions						
To the Strain	Depletion by strain		1:160	1:320	1:640	1:1280	1:2560	1:5120	1:10240
PR8	PR8	PR8	+++	+++	+++	+++	+++	+++	+++
PR8	Shklyaver	PR8	—	+++	+++	+++	+++	+++	+++
Shklyaver	PR8	Shklyaver	—	+++	+++	+++	+++	+++	+++
Shklyaver	Shklyaver	Shklyaver	+++	+++	+++	+++	+++	+++	+++
PR8	Physiological solution	PR8	—	—	—	—	—	+++	+++
Shklyaver	Physiological solution	Shklyaver	—	—	—	—	—	+++	+++

Key. /// positive reaction that is, no inhibition of hemagglutination; -hemagglutination-inhibition reaction.

Table 32

Crossed Neutralization Reaction of Strains PR8 and Shklyaver in Experiment on Eggs

Serum to strain diluted to 1:16 titer	Virus	Virus Dilution				Virus titer in presence of serum
		10^{-4}	10^{-5}	10^{-6}	10^{-8}	
PR8	PR8	0/4	0/4	0/4	0/4	1:10 000
PR8	Shklyaver	2/4	1/4	1/4	0/4	1:26 300
Shklyaver	PR8	1/4	1/4	1/4	0/4	1:10 000
Shklyaver	Shklyaver	0/4	0/4	0/4	0/4	1:10 000

Key. The numerator of the fraction is the number of eggs in the allantoic fluid of which a positive hemagglutination reaction was found: the denominator, the number of eggs in the experiment.

Table 34

Identification of New Virus Strains With Sera for A and B Strains in the Hemagglutination Reaction

Serum to strain	Animal	Titer	Hemagglutination–inhibition Reaction with Strains													
			S-6		Slb		Gavr		Lshcho		Yaks		PR8		WS	
			Titer	Ratio	Titer	Ratio	Titer	Ratio	Titer	Ratio	Titer	Ratio	Titer	Ratio	Titer	Ratio
A (PR8)	Sheep	160	10	0	1:20	1/8	1:10	0	1:10	0	0	0	1:160	1	1:20	1 8
A (WS)	Rat	1 280	10	0	1:40	1/32	0	0	1:20	1/64	0	0	1:160	1/8	1:1 280	1
A	Horse	640	20	1/32	40	1/16	1:20	1/32	1:10	0	1:20	1,32	1:640	1	1:320	1/2
B	Horse	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	Pig	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	Rat	80	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key. In the "titer" column the titer is indicated which is obtained in the hemagglutination–inhibition reaction, whereby the inhibition at a dilution of 1:10 is not taken into consideration in calculating the ratio of the heterologous to the homologous titer; in the column "ratio" the ratio is shown of the titers of the given serum in the reaction with the hetero–and homologous viruses.

Table 33

Antigenic Interrelationships Between Virus A (PR8) and Shklyaver Strains in Neutralization Experiment on Mice

Virus		Serum 1:10 to strain	No. of mice	In 10 days No. which: Sheep		Serum titers
Strain	Quantity			Lived	Died	
PR8	10	Shklyaver	5	5	0	
	100		5	5	0	
	1 000		5	5	0	Approximately
	10 000		5	0	5	5 000
	100 000		5	0	5	
Shklyaver	10	Shklyaver	5	5	0	
	100		5	5	0	
	1 000		5	5	0	Approximately
	10 000		5	5	0	50 000
	100 000		5	0	5	

In connection with the epidemic outbreak of influenza at the beginning of 1949 strains were isolated which did not fit within the framework of the two influenza A virus groups mentioned and which were also different from the B viruses. The relation of them to various typing sera is seen in Table 34. Here, it is fitting to note that the normal sera of the sheep, rabbit and guinea pig produced a nonspecific inhibition of the hemagglutination caused by these viruses in high titers (1:40, 1:320) whereas in the sera of rats and cocks no nonspecific inhibitors were presetn, or they were present in very low titers (no more than 1:10). Afterwards, the investigations were carried out only by means of rat and cock sera.

The new strains constituted a single serological group, which was shown when immune sera were prepared to previously existent and newly isolated strains. Fig. 70 gives us a distinct idea concerning these differences. At the beginning of 1951 influenza A-prime strains were obtained from abroad (the A-Sweden 3-50 and A-Sweden 6-50 strains), which made it possible to confirm the previously expressed idea that the 1949 strains belonged to the A-prime virus group.

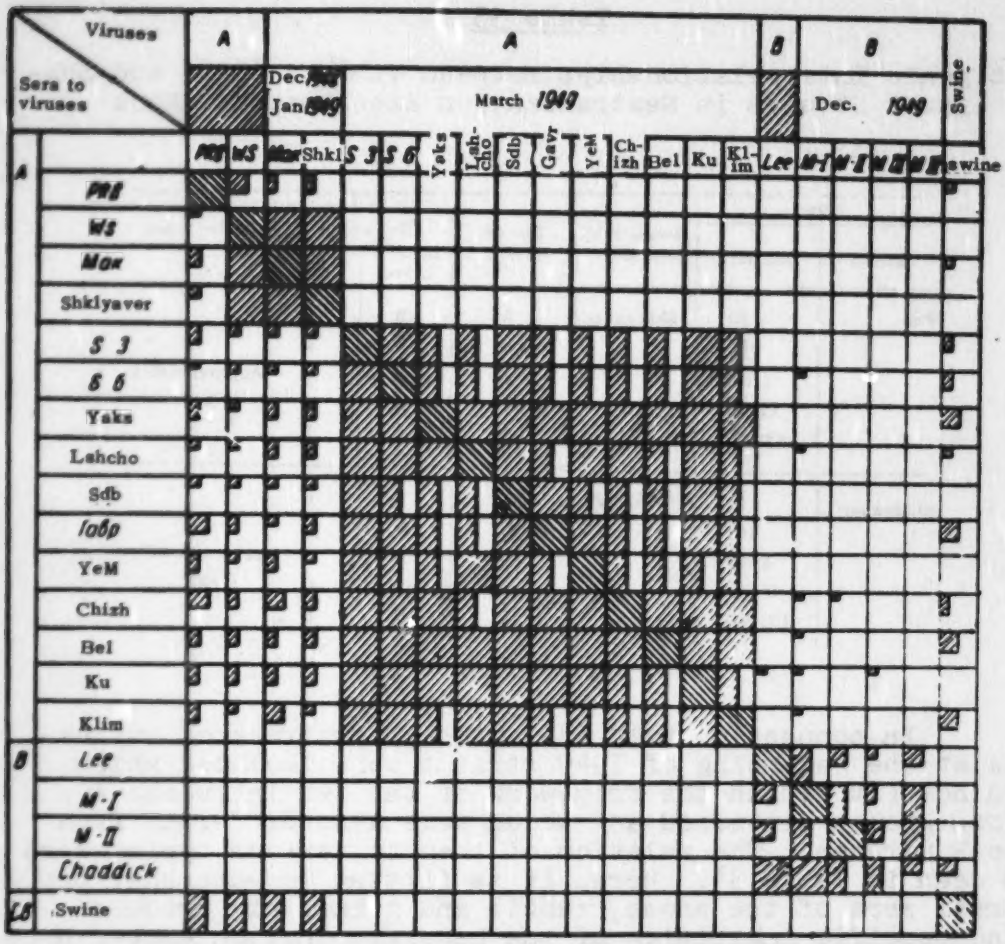

Fig. 70. Schematic Representation of the Similarity and Difference Between Strains of Various Groups of Influenza Virus.

Key is the same as for Fig. 69.

In Table 35 the results are presented of the investigation of Soviet and Swedish strains indicating that they belong to the A-prime virus group.

Somewhat later (in 1952) a standard foreign strain FM1 of the A-prime virus group was obtained with which the A-prime virus strains isolated in the USSR during the period from 1949 through 1952 were also compared.

Simultaneously, a similar study was made of the antigenic structure of the virus B strains as well as the strains intermediate between A and A-prime, the significance of which will be discussed in the next chapter.

Table 35

Identification of Soviet and Swedish A-prime Strains in Cross Hemagglutination-Inhibition Reactions with Their Antisera

Serum	Strains of viruses							
	Lshcho	Klim	S3	S6	PR8	Shklyaver	Swine	Kri
Lshcho	1	1	1	1	0,06	0,06	0,03	00
Klim	0,25	1	0,8	0,75	0,06	0,06	0,04	00
S3	0.5	0,6	1	1	0,05	0,06	0,02	00
S6	0.5	0,6	0,75	1	0,06	0.06	0.03	00
PR8	0	0	0	0	1	0,1	0	00
Shklyaver	0	0	0	0	0,1	1	0	00
Swine	0	0	0	0	0,06	0,03	1	00
Lee	0	0	0	0	0	0	0	10,2
Kri	0	0	0	0	0	0	0	0,41

Key. The decimals represent the ratio of the heterologous titer of a given serum to its homologous titer; the units represent thehomologous serum titer or the ratio wherein the heterologous titer is equal to the homologous titer; zero represents the ratio of titers amounting to a fraction less than 0.01 or a complete absence of the hemagglutination-inhibition reaction of the heterologous virus in a dilution of 1:10.

On the basis of these data extensive investigations were undertaken of the influenza virus strains for the purpose of working out a classification of them. One hundred and forty-five strains of influenza virus, including 102 strains isolated by workers of the department of influenza of the Institute of Virology of the Academy of Medical Sciences USSR and 30 strains isolated in other laboratories in various cities of the USSR (Moscow, Leningrad, Kiev, Khar'kov, Tashkent and others) and 13 strains obtained from foreign laboratories, were collected and studied from 1948 through 1954. The main investigations were accomplished by Gorbunova; she constructed a catalog of 915 strains described in the USSR and foreign countries from 1933 through 1952 inclusive, and she made a classification of them.

A standard method of recording the results of the hemagglutination-inhibition reaction was made the basis of the serological classification of influenza virus strains.

The nature of their serological relationships is established by means of comparing the strains in cross hemagglutination-inhibition reactions. These relationships may reflect slight antigenic differences between similar strains and also serve as a sign of the cardinal differences between groups of strains.

It has been suggested that the serological relationships be divided into intragroup and intergroup. The intragroup relationships characterize slight antigenic differences between the strains of the same serological group; the intergroup relationships are common to the entire group of influenza viruses, as a result of which they serve as themost substantial characteristics which make it possible to distinguish theprincipal groups of influenza viruses from one another.

Here we should like to present several examples characterizing the different natures of the antigenic relationships.

1. The reciprocal relationship of the cross reaction with the same indices, equal to unity or to a fraction. Unity indicates the complete identity of the strains, for example, PR8 and S6; the fraction indicates the same degree of similarity in the sense of the same degree of sensitivity of the strains to the neutralizing effect of their heterologous antisera (for example, strains PR8 and WS).

2. The reciprocal relationship of cross reactions with different indices of antigenic relatedness, which indicates a greater sensitivity of one of the two strains being compared to the neutralizing effect of antisera against the other strain with a more pronounced resistance of the second strain to antigens against the first strain. The Kri and Lee strains may serve as an example of such interrelationships.

3. Unilaterality of the crossed reactions, where the antibodies against one strain neutralize the hemagglutinating activity of another to some degree, whereas the antibodies against the second strain are completely incapable of neutralizing the hemagglutination produced by the first strain. As an example the relations between the A-prime strains isolated in the USSR in 1949-1952 and the A strains may be given.

In accordance with these three types of antigenic relationships between the strains of influenza we have constructed Table 36 in which an attempt has been made to reflect the results of our main observations on the study of the antigenic structure of the influenza viruses. The diagram (Fig. 71) is an illustration of the figured material which characterizes the distinctness of intra- and intergroup relationships among various representatives of the

influenza virus group.

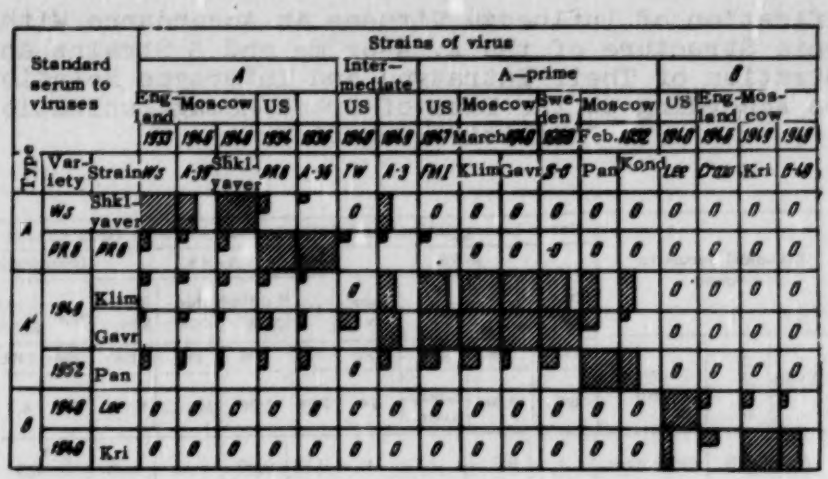

Fig. 71. Serological Classification of Influenza Viruses. The key here and later is the same as for Fig. 69 (Gorbunova, 1953).

A detailed analysis of the data presented in the Table and in the Figure should serve as material for making a scientifically grounded classification of influenza viruses.

Table 36 was made on the basis of results obtained in cross hemagglutination-inhibition reactions in accordance with our concept of the existence of influenza viruses of all three serological types, A, A-prime and B, in the USSR.

In the left half of the Table information is presented concerning the typing standard sera which belong to the main groups of Soviet virus strains which we studied experimentally in accordance with our data to the effect that viruses of the serological type A are divided into two varieties, WS and PR8. We have presented the results of experiments with standard Shklyaver (WS) and PR8 sera in this Table, and these results characterize the A viruses in an exhaustive manner. The A-prime group is represented by four standard sera against strains isolated in the USSR, whereby the data obtained with the Klim and Yaks sera have been combined into the same column since they supplement each other, being representatives of the same group of strains isolated in March 1949. The A-prime serum against the Gavr (March 1949) strain constituted a special standard,

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Table 36 [Left Half]

Classification of Influenza Viruses in Accordance With the Antigenic Structure of the A, A-prime and B Strains and a Demonstration of Their Intratype and Intertype Relationships According to the Data of Cross-Hemagglutination-Inhibition Reactions

Standard serum to viruses			Cross hemagglutination inhibition reaction with virus strains									
			A-WS				A-PR8				Intermediate	
			England	Moscow		Khar'kov	US	Leningrad	Moscow	Khar'kov	US	Leningrad
Type	Variety	Name of strain	1933	1946	1948	1952	1934	1936	1948	1949	1940	1949
			WS	A 39	Shklyaver	6-0	PR8	A 36	MS 12	Smor	TW	A 3
A	WS	Shklyaver	1	0,5	1	1	0,13	0,06	0,13	0,06	0	0,3
—	PR8	PR8	0,13	0,06	0,13	0,13	1	1	1	1	0,07	0,04
A_1	1949	Klim, Yaks, Gavr	0,08	0,06	0,08	0,06	0,03	0,03	0,03	0,08	0	0,4
			0,03	0,06	0,03	0,06	0,15	0,1	0,13	0,13	0,25	0,5
	1952	Pan	0,13	0,1	0,09	0,13	0,08	0,13	0,06	0,06	0	0,13
B	1940	Lee	0	0	0	0	0	0	0	0	0	0
	1949	Kri	0	0	0	0	0	0	0	0	0	0

because by means of this serum it was possible to detect a common component in typical and atypical A strains (PR8 and TW) isolated in the United States of America in 1934-1940 and strains of an intermediate group (A3 and A9), isolated in the USSR in 1949 during the period when the A-prime virus was becoming established in the Soviet Union.

A fourth A-prime serum (Pan) represents a standard which characterizes the antigenic structure of the 1952 strains.

Group B is represented by two varieties. This is serum against the well known Lee strain (USA, 1940) and Kri strain serum which has been accepted as a standard for the identification of B virus strains isolated in the USSR in December 1949.

Making use of these sera as standards we considered it possible to divide the strains which we studied into three groups -- A, A-prime and B -- with the inclusion of

a small transitional group of strains which we located between the two characteristic types -- A and A-prime.

Table 36 (continued)
[Right Half]

Cross hemagglutination inhibition reaction with virus strains																	
Intermediate	A, 1947–1950							A, 1952					B				
Leningrad	US	Moscow			Leningrad		Sweden	Moscow				US	England	Moscow		Leningrad
1949	1947	March 1949			April 1949		1950	February 1952				1940	1946	December		1949
A 9	M 1	Klim	Yaks	Gavr	AC 1	3 657	S 6	Pan	Avt	Krav	Kond	Lee	Craw	Kri	TS	B 49
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0,06	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0,3	0,75	1	1	1	1	1	0,75	0,5	0,25	0,8	0,4	0	0	0	0	0
0,5	1	1	0,5	1	0,75	0,5	1	0,25	0,06	0,5	0,06	0	0	0	0	0
0,13	0,25	0,25	0,06	0,13	0,13	0,13	0,25	1	0,5	0,75	0,5	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	0,13	0,13	0,13	0,08
0	0	0	0	0	0	0	0	0	0	0	0	0,38	0,25	1	0,75	0,6

Key. Unity represents the homologous serum titer or the heterologous serum titer equal to the homologous titer (identity of strains); the decimal represents the relationship of the heterologous to the homologous titer, showing different degrees of relatedness (from 0.75 to 0.25) or nonrelatedness (from 0.13 to 0.03) of the strains being compared; zero represents a complete absence of similarity or a ratio expressed by a number less than 0.03.

In proceeding with the analysis of the various groups of influenza viruses we should like to make the reservation that in Table 36 only certain exemplary strains of each serological type are represented which we studied in cross hemagglutination-inhibition reactions. We have inserted strains into the Table which have been isolated by various investigators, chiefly Soviet.

In Table 37 a list of strains was presented which were studied in our laboratory experimentally and identified

by means of a set of standard sera. We studied 13 strains of the WS variety and 10 strains similar to PR8, which belong to the serological type A; for atypical strains along with transitional strains from A to A-prime; 28 strains of A-prime belonging to the variety 1947-1950; and 15 strains of A-prime 1952; 10 strains of type B and one strain of swine influenza virus. Therefore, 80 strains of human influenza virus and one strain of swine virus were studied in the cross hemagglutination-inhibition reaction and by other methods. The viruses of human influenza could be classified according to the accepted categorization into three main groups -- A, A-prime and B -- and could be classified under one of the varieties mentioned.

Table 37

Distribution According to Groups of Strains Studied Experimentally

A(WS)	A(PR8)	Intermediate	A-prime 1947-1949-1951	A-prime 1952	B Lee	B Kri	Swine
WS,Mak	PR8 (obtained in (1940)	A3-A9	S3, S6, Yaks, Sdb, Lshcho	Pan,Avt	Lee (museum)	Kri, S8	1-220
Shkly-aver	PR9 (obtained in 1951)		Gavr, YeM, Chizh, Bel, Ku	Kond, Lom	Lee (obtained in 1941)	Laz, TS	
Sher, A39	A36, MS12		Klim, Volk, AS-1	Liga, Es	Lee (obtained in 1952)	B 49, Chaddick Goodloe, Crawley	
No 3, 7	Volk (A) Smor		A183 T1-49, 3,600	Krav, Yerm			
No 14, 3P	4-51, 5-51,		3710, 3711, 3632, 3642, 3657	Bay, Yur			

Table 37 [continued]

A(WS)	A(PR8)	Inter-mediate	A-prime 1947-1949-1951	A-prime 1952	B Lee	B Kri	Swine
61, 1L	7-51, Tr-1952		3699, 3656, 6418, 6540				
5K-60			No 1, Dutch vaccine	05/52 L7, Gor			
				A-Sweden -3/50			
				A-Sweden -6/50			

Afterwards, the list of influenza viruses studied was considerably supplemented by Soviet and foreign strains.

Returning to Table 36, it should be pointed out that the intragroup and intergroup relationships, which are determined by characteristics of the indices of antigenic relationship between the strains in a crossed hemagglutination-inhibition reaction, are the characteristics by which the serological groups of strains are generalized and particularized.

Group A (Serological Type A). Group A is represented by two varieties: WS and PR8. We used Shklyaver and PR8 antisera as the standard sera. The strains of the WS variety are represented by the following; S (England, 1933), A39 (Moscow, 1946), Shklyaver (Moscow, 1948) and 6-0 (Khar'kov, 1952). The viruses of the PR8 variety are represented by the following strains; PR6 (United States of America, 1934), A36 (Leningrad, 1936), MS12 (Moscow, 1948), Smor (Khar'kov, 1949). In the reactions with standard Shklyaver serum three strains of the WS variety (WS, Shklyaver, 6-0) show a complete correlation with its homologous titer, and one strain, A-39, reacts up to half titer. In accordance with this, the squares shown in Fig. 71 with figures of 1 and 0.5 are shaded in completely and to half of their extent respectively. The group of strains of the WS variety mentioned reacts in three cases to the extent of 1/8 and in one case (A39) to 1/16 of the serum titer of a PR8 strain, which belongs to another variety of the serological type A. These four strains (WS, A39, Shklyaver, 6-0) are neutralized to varying degrees in the hemagglutination reaction with sera against virus A-prime of both varieties

(1949 and 1952), namely, from 1/32, 1/16 to 1/8 of the titer of these sera. At the same time, both standard B sera (Lee and Kri) do not exert any inhibitory effect on the hemagglutination reaction of these strains.

The PR8, A36, MS12, Smor and the PR8 varieties of the same A type react to full titer only in the reaction with standard PR8 serum. Sera of the WS type, A variety and all the type A-prime sera, inhibit the hemagglutination reaction and these strains to the extent of 1/8-1/16 of their titer, whereby the Klim and Yaks antisera are less effective with respect to the strains of the PR8 group than the Gavr serum, although all these sera belong to the same group of strains, isolated in March 1949. In the same way, the group of strains which belong to the WS variety and the group PR8 strains are not neutralized in the hemagglutination-inhibition reaction of serum B (Lee and Kri).

Groups of strains belonging to the serological type A enter into crossed hemagglutination-inhibition reactions in such a way that the strains of the WS variety are neutralized by PR8 serum to the extent of 1/8 to 1/16 of its titer, just as strains of the PR8 variety are neutralized by Shklyaver (WS) serum to the same degree. This type of cross reaction characterizes intragroup relationships between the varieties of the serological type A, which can be designated as reciprocal (reciprocal relations of reactions within limits of 1/8-1/16 of the serum titers).

We shall make an analysis of the transitional group of atypical strains after we analyze the A-prime strains which are of particular interest to us.

Group A-prime (Serological Type A-prime). Separation of the serological group of A-prime strains into a special type of independent influenza viruses is based on the following interrelationships. First of all, we divided the A-prime strains into two varieties: strains isolated in March 1949, the standard sera for which were Klim, Yaks and Gavr sera, and the strains isolated in February 1952, the standard serum for the identification of which was the Pan serum. In accordance with the distribution in Table 36 the group of A-prime strains which had been isolated from 1947 through 1950 in the USSR and abroad was unified. As is well known, the A-prime strains were first found in the USSR in 1949; prior to this they had spread in the United States in 1947 and then in European countries. We had the foreign strains FM1 (United States of America, 1947) and A-Sweden 6/50 (Sweden, 1950) at our disposal; the Klim, Yaks, Gavr (Moscow, 1949), AS1, 3657 (Leningrad, 1949) strains are shown in Table 37 are representatives of A-prime strains 1949 isolated in the USSR. These seven strains have the nature of their crossed reactions with 1949

sera in common (Klim, Yaks and Gavr), showing inhibition of the hemagglutination reaction to half titer or to full titer in a mixture with these sera. As is seen from Fig. 71, the squares which correspond to the data for these reactions are shaded completely or are three-quarters or one-half shaded in accordance with the numerical expression 1; 0.75; 0.5. The hemagglutination-inhibition reactions performed with these 1947-1950 strains and with standard Pan serum give us an idea of the less pronounced correlation of heterologous titers of the serum with its homologous titer, which is demonstrated by the figures 0.06, 0.13, 0.25, corresponding to 1/16, 1/8 and 1/4 of the Pan serum titer.

At the same time, the 1952 Pan, Avt, Kond strains react to a lesser degree with sera of the 1949 standard (Klim, Yaks and Gavr) than with Pan serum (1952). Thus, inhibition of the hemagglutination reaction of the viruses mentioned by 1949 sera is defined by the limits of the relationship 0.5-0.06, whereas standard Pan serum inhibits the hemagglutination reaction of these viruses within limits of 0.5-0.75 of its titer.

At the same time, through the example of the Krav virus with 1949 and 1952 sera it should be noted that in 1952 viruses were isolated which had the same degree of similarity to both serological A-prime varieties in accordance with the numerical relationship 0.8-0.5-0.75 in the hemagglutination-inhibition reaction of Krav virus with Klim, Yaks, Gavr and Pan sera. These data reflect the intragroup relationship between the A-prime 1949-1952 strains, indicating their relative polymorphism.

Afterwards, a third variety of A-prime virus (1953) was isolated and studied. However, the intergroup relationships between the A-prime viruses and the A viruses are of special interest. In Fig. 71 zeroes are used to designate the reactions between all the A-prime virus strains (1949-1952) and the sera of both A (WS and PR8) varieties. At the same time, the A-prime sera (1949-1952) exert a notable neutralizing effect in the hemagglutination reaction on all the A strains belonging to both varieties. The limits of neutralization of strains of the earlier vintage (type A) by sera of the new type of virus range from 1/32-1/8 of the A-prime serum titers.

The intergroup relationships between A and A-prime viruses may be designated as unilateral with a complete absence of any reciprocal relationship. The latter may be explained as a deviation of the new type of virus from the old type, by the acquisition of new antigenic properties with the preservation of the ancestral antigenic heritage in type A-prime and complete stabilization of the antigenic

structure in type A.

The earliest of the A-prime strains at our disposal, FM1, was isolated in the United States during the first great A-prime influenza epidemic in 1947, at which time the distinctness of the antigenic structure of the new serological A-prime strains was clearly established. Through our system of serological analysis by the comparative method the great similarity of the antigenic structure of the American FM1 1947 strain was noted to the Soviet A-prime strains isolated in March 1949. Thus, the FM1 virus is neutralized in the hemagglutination-inhibition reaction by sera against the Klim, Yaks and Gavr strains to the extent of 3/4 or full titer; the serum to the Pan strains (A-prime 1952) inhibits the hemagglutination reaction of FM1 virus to the extent of 1/4 of its titer, which is characteristic of strains circulating in the USSR in 1949. With respect to A-prime sera the FM1 strain shows a great similarity to the Swedish A-Sweden, 6/50 strain. However, through careful study of the FM1 virus the interesting property of this strain may be noted of entering into the hemagglutination reaction with PR8 serum to the extent of 1/16th of its titer. Inhibition of the hemagglutination reaction of strain FM1 is characteristic of only a single variety of the standard A sera, namely, PR8, but not Shklyaver (WS). This fact gives us a direct indication of the origin of the American strain (FM1) from strains of the serological type PR8, which, as is known, have been prevalent on the American continent since 1934. The subsequent evolution of A-prime becomes clearer if we compare FM1 with the March 1949 and Swedish 1950 strains. This comparison gives us the grounds for the assertion that the first strains of the new type (FM1) maintained their sensitivity to PR8 antibodies with a much more clearly manifested capacity of reacting with sera against similar strains of a later vintage (1949-1952), which had completely lost their sensitivity to antibodies of the type A influenza virus. The resistance of antibodies against the serological type A was established as a characteristic feature of the A-prime strains which had been isolated later, namely, in 1949 and 1952 in the USSR and in 1950 in Sweden.

If we draw a comparison between the FM1 (United States, 1947) and TW (United States, 1940-1941) strains, we find a further-reaching discrepancy between the serological characteristics. The sensitivity to the PR8 antibodies is the same in the TW and FM1 strains (0.07 and 0.06). However, the relationship to antibodies of sera against the A-prime strains (Klim, Yaks and Pan) is completely different.

Whereas, the TW virus shows a complete resistance

to the inhibiting effect of the hemagglutination reaction of the A-prime sera mentioned, the FM1 virus is neutralized by these sera within limits of titers characteristic of the A-prime 1949 strains (0.75; 0.25). If serum against the Gavr strain is substituted for sera against the Klim and Yaks strains in the hemagglutination-inhibition reaction, a greater similarity is found between the TW strains and the A-prime strains.

Therefore, we believe that the A-prime strains isolated in the USSR in March 1949 constitute an example of a further evolution of the A virus than the FM1 virus. The influenza FM1 virus may serve as an example of the incomplete formation of a new type, whereas our strains, beginning with 1949, and the foreign strains -- of later origin -- represent examples of the formation of a new type of influenza virus in a finished form. We distinguish A-prime as an independent serologic type resistant to antibodies against type A but possessing the property of stimulating antibodies which, to a considerable degree, neutralize the type A viruses. This preserved property, in a manner of speaking, shows the roots of origin of the A-prime viruses.

The beginning of the study of the A-prime strains in the USSR coincided with the isolation of Soviet strains in 1949 which are different from the YeM-prime strains in the complete absence of sensitivity to antibodies against type A. Therefore, it seemed to us at that time that the name A-prime was not entirely suitable for this independent group of strains. Only comparative experiments between the 1949 strains and the FM1 strains established the true significance of the A-prime strains in theprocess of evolutionary variation of the A virus and their subsequent evolution en route to the formation of a new serological type of influenza virus on the basis of the old one (see Chapter III).

Returning to the transitional group of strains presented in Table 36, mention should be made of strains A-3 and A-9 isolated in Leningrad in 1949 which, in contrast to the American TW and FM1 strains, represent a stage between the strains of the serologic variety circulating in the USSR and the newly formed A strains.

In accordance with the data presented in Fig. 71, type B sera do not exert any notable neutralizing effect on the A and A-prime viruses, just as the A and A-prime sera do not exert any effect on the B viruses.

Group B. Viruses of the serologic type B represent a group of strains defined by the absence of crossed reactions with A and A-prime strains. Therefore, the intergroup relationships of the serologic type B indicate a complete independence of these virus strains of the other two.

The intragroup relationships of the B strain are characterized by their cross reactions with sera gainst the standard Lee strain (United States, 1940) and with the serum against the Kri strain (USSR, 1949).

In accordance with the magnitude of the numerical relationships between the heterologous and homologous titers of these sera and viruses two groups of B strains are clearly indicated. These are the Lee strain, which is neutralized by serum against the Kri strain to the extent of 0.38 of its titer in addition to being neutralized by the corresponding Lee serum (titer taken as 1), and the Kri strain which in the cross hemagglutination-inhibition reaction with serum against the Lee strain is neutralized to only half the degree (0.16 of its titer) to which the Lee strain indicated above is neutralized by serum against the Kri strain.

It is of interest that serum against the Kri strain possesses a greater serological similarity than does serum against the Lee strain not only to strains isolated in 1949 in the USSR but also to the English Crawley strain of earlier vintage (1946) than the Kri prototype. Therefore, the Crawley strain is closer to the Kri group of strains than to the Lee 1940 strain.

Experience has shown that there are differences between the two groups of B strains but that they are much less pronounced than the differences between the PR8 (A) and FM1 (A-prime) strains. To this it should be added that we studied B strains isolated in three successive epidemics (1940, 1946, 1949), just as we had studied the A and A-prime strains (1934, 1947, 1949). However, the evolutionary variability of the A virus was shown in all evidence, whereas the variability of the B strains remains within type limits.

Crossing of the hemagglutination-inhibition reaction indicates the reciprocal nature of intragroup relationships, which excludes the question of the independent significance of these groups. Therefore, two Lee and Kri varieties exist among the B strains.

Trhough the example of the variability of the B virus, nevertheless, the same tendency may be observed toward the occurrence of a broader spectrum of antibodies in the antisera of strains of later vintage (Kri, 1949) than of the viruses circulating previously (Lee, 19 0), as was quite distinctly shown through the example of A-prime antiserum with respect to the A viruses. Such a trend of variability of the B virus gives us the grounds for assuming a further development of it in the future in the same manner as A and A-prime viruses.

In concluding the review of the principal antigenic

relationships between the influenza virus strains designed in accordance with an analysis of the intra- and intergroup relationships, we should like to note once again the value of the hemagglutination-inhibition reaction, by means of which the heterogeneity of the virus antigen may be evaluated quantitatively by the serum antibodies. By means of this reaction it is possible to show the specific nature of the evolution of the antigenic structure of the influenza virus and to reveal the sources of formation of the new serological virus type, as has been shown through the example of the A-prime influenza virus.

Therefore, we have approached the classification of influenza viruses from a phylogenetic standpoint, dividing the A and A-prime strains into two groups in accordance with the detection of the distinct nature of their intra- and intergroup relationships.

In Table 38 a classification is given of the serologic types (groups) of influenza viruses corresponding to the results of an analysis which was made schematically in Table 35 with the addition of the necessary information which has recently been obtained. Each of the serologic types (groups) A, A-prime and B is represented by two varieties of strains isolated during various epidemics by different, chiefly Soviet investigators. The names of the strains in the group pertaining to a certain variety are arranged in chronological order in accordance with the dates of their isolation.

Table 38

Serologic Classification of Influenza Viruses

Group (Serologic Type)	Variety	Strains		Reference to Literature	
		Year of Isolation	Name	Place of Isolation	Author
1	2	3	4	5	6
A	WS	1933	WS	England	Smith, Andrewes
		1946	A39	Moscow	Smorodintsev
		1948	Mak, Shklyver, Sher	Moscow	Gorbunova

Table 38 [continued]

Group (Serologic Type)	Variety	Strains		Reference to Literature	
		Year of Isolation	Name	Place of Isolation	Author
1	2	3	4	5	6
	PR8	1934	PR8	United States	Francis
		1936	A36	Moscow	Smorodintsev
		1948	MS12	Moscow	Fal'kovich
A'	FM1	1947	FM1	United States	Rasmussen and others
	Klim	1949	Klim, Yaks, Lehcho, YeM and others	Moscow	Gorbunova
		1949	AS1, A183 and others	Leningrad	Smorodintsev
		1949	3500,3642, 3656,3657, 3711 and others	Leningrad	Romanenko
		1949	T1-49	Tashkent	Maksianovich
		1950	A-Sweden 3/50	Sweden	Svedmyr
			A-Sweden 3/50		
		1951	6418,6540, 6541 and others	Leningrad	Romanenko
	Pan	1952	Pan, Avt, Krav, Bay, Yerm,Vas, and others	Moscow	Ritova
			Liga and others	Moscow	Zakstel'skaya
			Muks, Iva	Moscow	Fadeyeva
			Kond	Moscow	Sokolov and Kulikova

Table 38 [continued]

Group (Serologic Type)	Variety	Strains		Reference to Literature	
		Year of Isolation	Name	Place of Isolation	Author
1	2	3	4	5	6
			85-52, LP-52	Moscow	Solov'yev and Gutman
A''	A-Singapore -1/50		A-Singapore-1/50	Singapore	Hale
			A'' Moscow (Iksha, Ber, Chel and others)	Moscow and other cities of the USSR	Zhdanov, Ritova, Zakstel'skaya and others
B	Lee	1940	Lee	United States	Francis
	Kri	1946	Crawley	England	Dudgeon and others
		1949	Kri, S8, Laz, TS	Moscow	Grobunova
		1949	B49	Leningrad	Smorodintsev
		1949	4945	Leningrad	Romanenko
		1954	B55, Sych	Moscow	Solov'yev, Gutman
C'		1947	1233	United States	Taylor
		1950	JJ	United States	Francis
		1956	S-Moscow-1956	Moscow	Solov'yev, Gutman
D		1953	Sendai (Fusimi)	Japan	Kuroya and others
		1957	D-Vladivostok-56	USSR	Gerngross, Gorbunova
		1957	D-Moscow-57	USSR	Ustanov, Ritova and others
Swine		1931	Swine	United States	Shope

Aside from types A, A-prime and B, the C virus has been isolated as a special fourth serologic type including a total of two strains which have been described in the literature, JJ and 1233, as well as virus D.

A strain of this type, 1233, was isolated for the first time by Taylor (1949) in 1947 during an outbreak of A-prime influenza. In 1950, Francis isolated the JJ strain, identified it with the Taylor strain, and called it type C (Francis, Quilligan and Minuse, 1950). We studied representatives of this type of virus later. According to the data obtained, both type C strains are different from the other virus types not only in the characteristic nature of its antigenic structure but also in its other biologic properties. Thus, both strains agglutinate chick erythrocytes only in the cold without exerting any hemagglutinating effect on guinea-pig erythrocytes under any conditions. Information dealing with the epidemiological, biological and serological characteristics of the C virus are available in the reports of Taylor (1949, 1951), Francis, Quilligan and Minuse (1950), Davenport (1951), Hirst (1950) and Sol-ov'yev (1956).

The virus of swine influenza should be considered a fifth type. We are considering this virus along with the human viruses because there are data which indicate the genetic similarity of both viruses and even an identity of them.

This virus, which was isolated by Shope (1931) during an epizootic of swine in America, remains a riddle with respect to its etiologic connection with the influenza pandemic of 1918 and a number of epidemics in subsequent years up to the present time.

According to the data of Hudson, Siegel and Markham (1943), the viruses of swine influenza, including strains isolated in Britain, are closely related to the human A viruses but very much different from the B viruses.

The previous investigations by Francis and Magill (1936) concerning the antibodies existing in human sera against the virus of swine influenza have been confirmed by recent investigations of Kalter and others (1948). In addition, according to the report of Francis and others, Quilligan and Minuse (1949) the Oti strain of swine influenza virus isolated in Korea in 1939 has proved to be similar antigenically to the A-prime virus (Rhodes, FM1).

According to our observations, during work with the 1-220 swine influenza virus strain which we have in the Soviet Union we found that it was neutralized by many A-prime sera in the absence of crossed reactions, which indicates its similarity to A virus. We did not find any crossed reaction with the B strains.

Therefore, the serological classification of influenza viruses is supplemented by a fifth group of viruses which possess a distinct antigenic structure, which was revealed by an analysis of the intragroup and intergroup relationships between the strains.

The investigations of recent years, by confirming our ideas of the principles of classification of influenza viruses, have made it possible to introduce a number of corrections and additions.

First of all, this applies to the discœry of the new influenza D virus found in Japan (Kuroya and others, 1953) and then in the USSR (Gerngross, 1957; Gorbunova and others, 1957; Zhdanov, Ritova and others, 1957). Viruses isolated in various places (Japan, Vladivostok, Moscow) have been shown to be completely identical.

Material from the investigations of the pandemics of "Asiatic" influenza 1957 also permit us to make considerable corrections. The viruses isolated abroad and in our laboratory constitute the next step in the evolution of the A-A-prime series, whereby many strains have completely lost their antigenic relationship with the A viruses in the hemagglutination reaction, preserving it only in the complement-fixation reaction. Therefore, in this case we may speak of the formation of a new antigenic type of virus, A-double prime, as a member of the A virus series.

In the light of these data it seems better to us to speak of the A, A-prime and A-double prime viruses as subtypes or varieties of the A virus rather than as separate types.

It is fitting to compare certain investigations on the classification of influenza viruses abroad with the material presented, discussing chiefly the investigations of recent years. A part of these works will be analyzed in greater detail in the chapter on the variability of influenza viruses.

Hilleman (1952) distinguishes four main components (corresponding to the WS, PR8, FM1 and FM1-50 strains) in the group of A viruses, two components (Lee, Warner) in the group of B viruses, and one component in the group of C viruses (1233). Designating each type of virus with capital letters of the Latin alphabet and each component with small letters and the quantity of it in the strain by indices from zero to three, he suggests the following antigenic formulas for the prototype strains:

WS: $Aa_3b_1c_0d_0$	Lee: Ba_3b_0	1233: ca_3
PR8: $Aa_2b_3c_0d_0$	Warner: Ba_1b_3	JJ: Ca_3
FM1: $Aa_1b_0c_3d_3$	JB1: Ba_0b_3	

Wtg: $Aa_0b_0c_3d_3$
SF1-49: $Aa_0b_0c_1d_3$
PW1-50: $Aa_0b_0c_1d_3$

These formulae are of interest from the point of view of the presence of various antigenic groupings in the composition of the antigens of various strains, but they are hardly applicable to the classification of strains, because they constitute a poorly grounded attempt to classify influenza viruses by analogy with Salmonellae.

The same kind of attempt is found in the works of Jensen (1953) and Francis (1953), where the authors have devised 18 antigenic groupings among the A and A-prime viruses.

In contrast to these authors, Magill and Jotz (1952), studying 100 strains of A, A-prime and B viruses, found five groups among the A and A-prime viruses and four groups among the B viruses. It is characteristic that all these three works were accomplished with approximately the same or, in any case, a similar set of strains. Different conclusions which were obtained by the three groups of authors attest to the subjective and arbitrary approach to the problem of serological classification of influenza viruses.

It is quite obvious that in proceeding along this line it is impossible to work out a scientifically-grounded classification of influenza viruses, not to mention the fact that the serological principle, taken by itself but not in comparison with the phylogeny of viruses, can lead, and in the given case has led to chance results and was not a reflection of the objective rules and regulations of influenza virus variability.

From this standpoint it is very characteristic that none of the three suggestions has obtained the recognition of the majority of virologists, and in the official publication of the World Health Organization (Influenza, WHO monograph ser. No 20, Geneva, 1954) by Magnus (1954) a more natural classification of viruses is proposed, which to a considerable degree is similar to ours. The author distinguishes three immunologically independent types of influenza viruses: A, B and C. Within the limits of types A and B he distinguishes the main groups, designating them in accordance with the names of the prototype strains: WS, PR8, FM1 and FM1-50 for the type A viruses and, respectively, Lee and Bon for the type B viruses.

This classification is nearer to being correct than the previous ones, because it reflects more exactly the processes of influenza virus variability observed. At the same time, in comparison with the classification which we

have proposed its defects, as an empirical classification unsupported by generalizations of observed facts and not taking into consideration the processes of species formation among influenza viruses during the course of their natural evolution, are obvious.

29. Comparison of Biological Properties of Influenza Viruses of Various Types

In concluding the present chapter it seems expedient to give a comparative review of the biological properties of the influenza viruses.

The influenza viruses known at the present time differ from one another in their biological properties.

Briody (1950) considers the A viruses (in this group he includes A and A-prime strains) and B viruses different species according to the conglomeration of their biological properties and in support of this view he gives a table in which the principal properties of these viruses are summarized (Table 39).

Table 39

Comparative Data on the Biological Properties of A and B Viruses According to Briody (1950)

No	Properties	A	B
1	Antigenic structure	Different	
2	Periodicity of the epidemics	2-3 years	4-6 years
3	Immunity	Weaker	Stronger
4	Pathogenicity for laboratory animals	Stronger	Weaker
5	Isolation in ferrets	Easy	With difficulty
6	Duration of first growth cycle	5-6 hours	9-10 hours
7	Destruction of receptors	Less complete	More complete
8	Hemagglutination inhibition by normal sera	Weak	Strong (to high titers)
9	O-phase	+	-
10	H-phase	±	+
11	Size of elementary bodies	80-120 mμ	80-120 mμ
12	Temperature optimum of growth	35-37°	35°
13	Hemagglutinating properties	For a few species of animals	For many species of animals

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Table 39 [continued]

No	Properties	A	B
14	Stability of hemagglutinin to temperature and ultraviolet irradiation	Low degree	High degree
15	Optimum pH	6.5-7.0	7.9 or over
16	Resistance at temperature of -70°	+	-

Undoubtedly, certain of the data presented call for a critical attitude (for example the periodicity of the epidemics, which can in no way be taken as a property of the virus but rather depends on many other factors); however, in general this Table gives us an idea of the difference between the biological properties of A and B viruses.

Summarized data concerning the most essential differences between the influenza viruses of various types are presented in Table 40.

Table 40

Comparison of Properties of Influenza Viruses A, B, C and D

Properties	A		B	C	D
	Type A	Type A'			
Size in mμ	80-90	80-90	80-90	80-90	80-90
Pathogenicity for mice	Primary	Requires adaptation	Requires adaptation	Does not adapt	Primary
Multiplication in chick embryo	In all the cavities. Original isolation preferable in the amnion.				
Optimum growth temperature	37°	37°	35°	34°	37°
Toxicity for mice	Primary	After adaptation	Primary (partial)	Not toxic	Primary
Hemagglu-					

Table 40 [continued]

Properties	A		B	C	D
	Type A	Type A'			
tination of chick erythrocytes	Present	Present	Present	Possible at low temperatures (+ 4°)	Present
Hemagglutination of guinea-pig erythrocytes	+	+	+	-	+
Group antigens	S-antigen common; hemagglutinin partly common to both		Specific	Specific	Non-specific
Cytopathogenic effect on human fibroblasts	-	-	-	-	+

It is known that the A-prime viruses adapt more rapidly to chick embryos than the B viruses, in connection with which the latter are isolated with greater difficulty from throat washings. The B viruses are readily passaged in cultures of human tissue. In human tissues the A-prime viruses multiply during active cell growth, whereas the B viruses multiply better at 35° when the tissue is growing only slightly.

As far as the C virus is concerned, according to Taylor's data (1951), in size and morphology it is similar to the A and A-prime viruses. In the neutralization, hemagglutination-inhibition and complement-fixation reactions as well as in experiments with crossed infection of hamsters and vaccination of people it is different from the A, A-prime and B viruses. The virus develops well in the amnion and not so well in the allantois. Ferrets and hamsters show a rise in antibody titer without signs of the disease after intranasal infection; the infection does not develop in mice after intranasal or intracerebral infection. The virus elutes rapidly from the erythrocyte at room temperature; therefore, in contrast to other influenza pathogens

the hemagglutination reaction with C virus must be performed at 4°. In man, the C virus produces milder diseases than the other viruses, and most often infection with it is accompanied by the development of an asymptomatic infection. Among the characteristics of this virus is also the fact that inhibitors of normal sera do not affect the hemagglutination produced by the C virus.

To date, the C virus has been isolated in sporadic influenza-like diseases or during small outbreaks; there have been no large epidemics of C influenza recorded. At the same time, large-scale investigations of human sera in the United States have shown that in 62.2 percent of persons investigated there were antibodies against the type C virus in titers of 1:64 and higher, whereby in contrast to the A, A-prime and B viruses, the high antibody titers to C virus were observed not only in adults but also in children over three years of age (Hilleman, Werner and Gauld, 1954). During an outbreak of A-prime influenza in 1956 and with the examination of the antibody rise among 900 paired sera an increase in antibodies to the virus was found in 6.6 percent of the patients (Solov'yev, 1956). As far as the D virus is concerned, it has been inadequately studied as yet. Its existence has been recorded in a few countries.

The comparative data presented are to a certain degree schematic, because they do not take into consideration the variability of influenza viruses, which will be analyzed in the next chapter.

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THIRD CHAPTER

VARIABILITY

30. Changes in the Antigenic Structure of the Influenza Virus During the Epidemic Process

Soon after the discovery of influenza viruses investigators occupied in the study of it ran up against the marked variability of the virus strains both during the process of isolation of them and during the course of subsequent work with them in the laboratories. While they do not constitute an exception among the viruses, which, like any other living thing, are characterized by the phenomena of variability, the influenza viruses are at the same time distinguished from many other viruses by a particularly pronounced plasticity.

The variability of the influenza viruses observed pertains to different biological properties: the antigenic structure, the immunogenic properties, the pathogenicity for laboratory animals, toxic properties, tissue tropism, hemagglutinating activity, rate of multiplication, morphology, biochemical activity, resistance to varius environmental factors, etc.

Although many of these properties are closely associated with one another and are interdependent, it is expedient to analyze individually the elements in the factual material which pertain to various aspects of variability of influenza viruses.

Soon after the discovery of the influenza A and B viruses attention was directed to the fact that the virus strains isolated were different from one another in their antigenic structure.

Usually, strains isolated from different patients during the same outbreak are identical in their antigenic structure. The observations of Andrewes, Laidlaw and Smith (1935), who during the influenza epidemic in England in 1935 isolated eight strains of A virus identical with one another and similar to the WS strain, are evidence of this. Taylor and Dreguss (1941) investigated 16 strains of A virus

isolated during the 1939 epidemic in Poland, Czechoslovakia and Hungary; they were all identical with one another, and were quite similar to PR8 (neutralization to 1/5-1/125 titer) and were considerably different from the WS strain (neutralization of 1/125-1/625 titer). Clampit and Gordon (1937) isolated nine strains identical with one another and similar to the PR8 virus during the outbreak of influenza in December 1936 in the United States. The A virus strains isolated by Beveridge and others, (1944) in Australia during the interepidemic period in 1943 were similar to one another and to the PR8 virus.

There are observations according to which the strains of virus isolated during the same epidemic differed from one another in their antigenic structure. Burnet and Lush (1940) isolated five strains during an outbreak of influenza in Australia in 1939 which were related to the A PR* variety; however, the antigenic differences between them were so pronounced that the authors considered it possible to divide them into three groups. Finland and others (1945) isolated strains similar to the Lee virus during an epidemic of influenza in the United States at the end of 1945, and after the epidemic isolated strains which had a different antigenic structure.

All these differences are of lesser importance than the completely distinct tendency toward changes in the antigenic structure of influenza viruses which occurred everywhere in chronological sequence.

Observations indicating a difference in antigenic structure of the influenza virus strains of one type isolated during various epidemics have been described previously also (Andrewes, 1942; Hirst, 1947); however, only during recent years have these data attracted attention.

Chronological Sequence of Occurrence of Antigenic Varieties. If we compare the time of isolation of the influenza virus strains in various countries with their antigenic characteristics a very distinct picture is obtained of a progressive change in the antigenic structure of influenza viruses. In Figs. 72-74 data are presented concerning the antigenic characteristics of influenza viruses isolated in various countries from 1934 through 1954. The viruses are distributed in accordanced with the types and subtypes of the Gorbunova's classification system.

Study of these data makes it possible to represent the changes in the antigenic structure of the influenza viruses in a generalized way and in the following form.

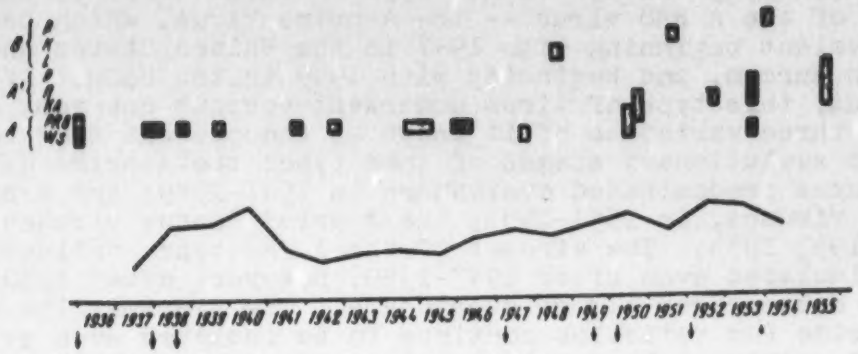

Fig. 72. Influenza Morbidity Rate in the USSR and the Characteristics of the Strains Isolated.

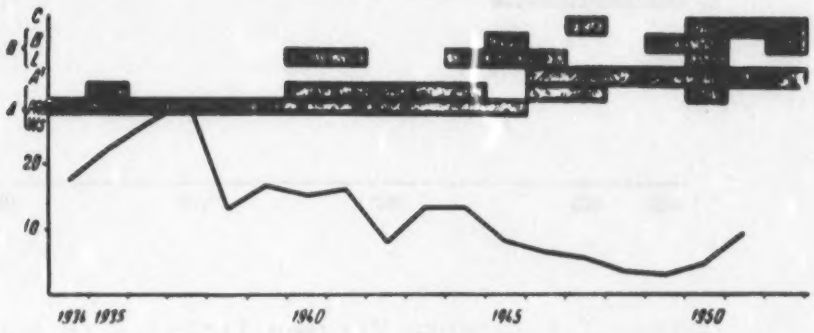

Fig. 73. Mortality Rate from Influenza in the United States and Characteristics of the Strains Isolated.

During the course of 1933-1936 strains of the A WS type and related strains were predominant among the influenza A virus. Beginning with 1936, first in the United States and then in other countries, the main type of A virus began to be the A PR8 strain, although in the USSR and European countries prior to 1941-1942 quite often and then much less often the strains of the previous type continued to be isolated. Strains of the A PR8 type gradually changed their antigenic structure from year to year, particularly between 1941-1945, when strains with new antigenic groups

appeared (Australia, Scandinavia, United States of America). Between 1945 and 1947 a new type of virus arose on the basis of the A PR8 virus -- the A-prime virus, which became prevalent beginning with 1947 in the United States and Western Europe, and beginning with 1949 in the USSR. Afterwards, this type of virus underwent various changes, and the three varieties of it known at the present time represent evolutionary stages of this type: the A-prime Klim viruses predominated everywhere in 1947-1949; the A-prime Pan viruses, in 1951-1952; the A-prime Rostok viruses -- in 1953 1954. The viruses of the A PR8 type continued to be isolated even after 1947-1949; however, after 1952 they had disappeared almost everywhere. The A-prime Klim and A-prime Pan varieties continue to be isolated even at present, although they are no longer the dominant types of A-prime viruses.

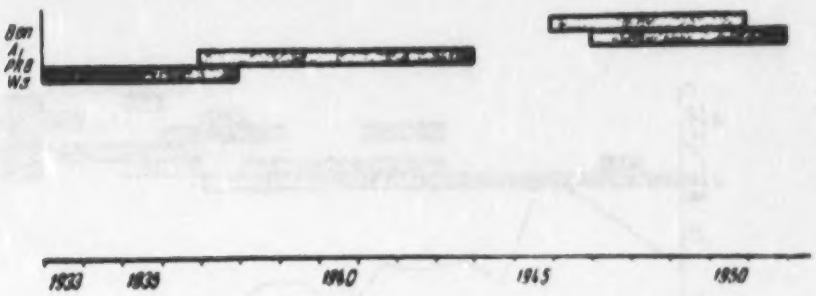

Fig. 74. Types of Influenza Viruses Isolated in England in 1933-1951.

The considerations presented, which we stated in 1949, were afterwards given a firm basis in a number of subsequent works (Zhdanov, 1950, 1951, 1952, 1953, 1954; Zhadanov and Solov'yev, 1951, 1952). In Fig. 75 this process of variation of the influenza A-A-prime viruses has been represented schematically, and in Fig. 76 the data on the antigenic structure of typical strains of Influenza A-A-prime viruses isolated in Moscow during the period from 1948 through 1953 are shown. Previously, in the second chapter, the serological classification of influenza viruses has been presented as worked out by Gorbunova. A comparison of the data just presented with this classification permits us to come to the conclusion that the latter is con-

clusion that the latter is constructed on a phylogenetic basis and reflects the evolution of influenza viruses observed during the epidemic process.

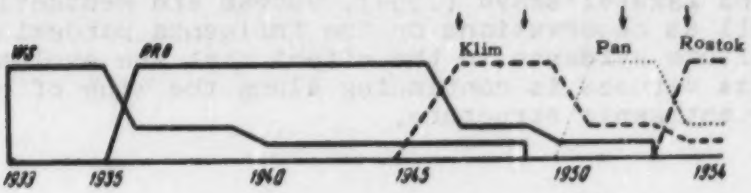

Fig. 75. Antigenic Structure of Influenza A and A-Prime Viruses for 1954.

The arrows indicate the years of epidemic outbreaks of influenza A and A-prime in the USSR.

Strain		Serum			
Name	Year of isolation	Shkiyaver	Klim	Pan	Rostok
Shkiyaver A	1948		0	0	0
Klim A'	1949				
Pan A'	1952				
Rostok A'	1953	0			

Fig. 76. Antigenic Structure of Influenza and A-Prime Viruses Isolated in Moscow From 1948 Through 1953.

The hatched part of the square corresponds to the fraction obtained by dividing the heterologous serum titer by the homologous serum titer; zero represents a ratio of 1/64 or lower; a completely hatched square corresponds to a homologous titer taken as unity or to the ratio of the heterolo-

gous titer to the homologous titer when they coincide; n/p -- experiment not performed (Zhdanov, 1954).

The subsequent observations and investigations of the antigenic structure of influenza viruses made in the laboratories under our direction (Zhdanov, Solov'yev) Ritova and Zakstel'skaya (1954), Gutman and Mentkevich (1954) as well as observations on the influenza pandemic in 1957 constitute evidence to the effect that the evolution of influenza viruses is continuing along the line of change in their antigenic structure.

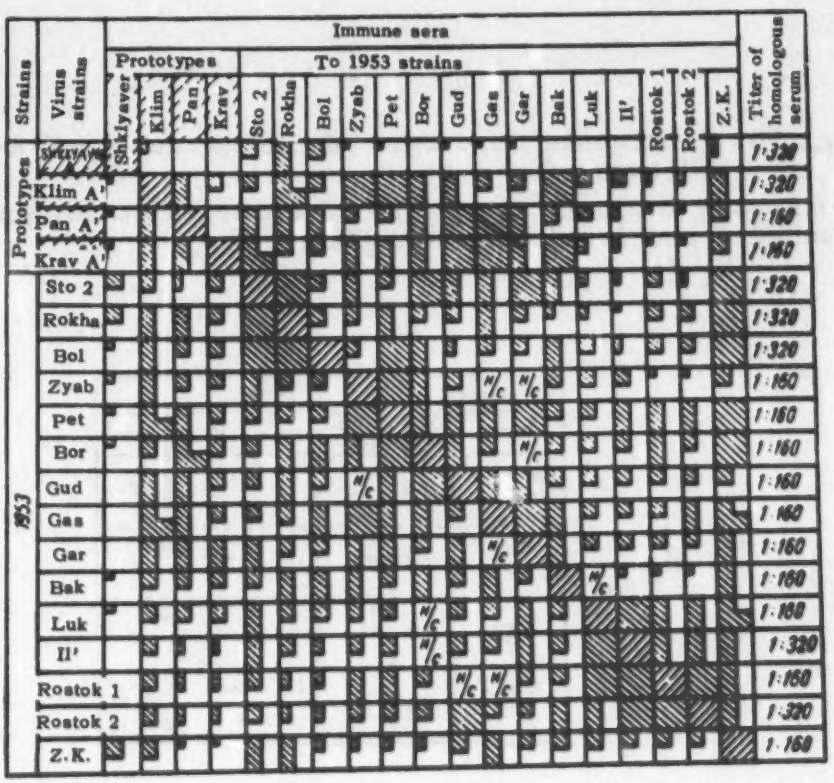

Fig. 77. Antigenic Structure of A-Prime Virus Strains Isolated in 1953 (Ritova, 1954).

The key is the same as for Fig. 76.

Ritova and Zakstel'skaya in 1952-1954 isolated and studied the antigenic structure of 45 strains of A-prime influenza virus and one strain of B virus. The strains of A-prime virus isolated in 1952 (13 strains) belonged chiefly to the Pan variety of the A-prime virus, although they were well neutralized by sera against the Klim variety and in general represented a quite homogeneous group.

The strains of the A-prime virus isolated in 1953 (32 strains) were less homogeneous (Fig. 77, 78), and in their antigenic characteristics they can be divided into three groups. The first group was comprised of strains (Rokha, Stog, Gup and others), which, while they were undoubtedly A-prime viruses, at the same time contained a part of the antigens of the A virus. The second group was comprised of viruses (Zyab, Gud, No 6 and others) which, while similar to the Klim and Pan A-prime virus, showed no or only a very slight antigenic similarity to the A virus. The third group was made up of viruses (Rostok, Luk, No 1 and others), which, while they were A-prime viruses, were notably different from the varieties of this virus isolated in 1949 and 1952 (Klim, Pan), although they showed a considerable antigenic similarity to them. The B virus strain (Lev) was similar to the Kri variety and was considerably different from the Lee variety.

Similar data have been obtained by Gutman and Mentkevich who in 1953 isolated and studied 11 strains of A-prime viruses. As seen from Table 41, these strains, which are similar in their antigenic structure to the viruses isolated in 1949 and 1952, were at the same time notably different from them.

A similar phenomenon occurs also in connection with the B viruses. From 1940 through 1945 B viruses isolated in various countries were identical or similar in their antigenic structure to the Lee prototype strain. Beginning with 1943 in Australia and then in other countries strains of a new variety began to appear -- of the B-Kri type (Bon). As early as 1945 they began to be predominant among the B viruses, although the preceding variety continued to be isolated until 1950. The strains described by Romanenko and Fridman (1954), which they had isolated in Leningrad in 1952, should be regarded as a new variety -- the B-Rom virus. Therefore, the process of variation of the B viruses is of the same progressive nature as that of the A-A-prime viruses but occurs more slowly and in the interval of time it has been observed it has not as yet led to the formation of the B-prime virus.

As far as the C and D viruses are concerned, the strains which have been isolated up to now are not much different from one another in their antigenic structure, and,

by the way, these viruses and the diseases caused by them have been inadequately studied.

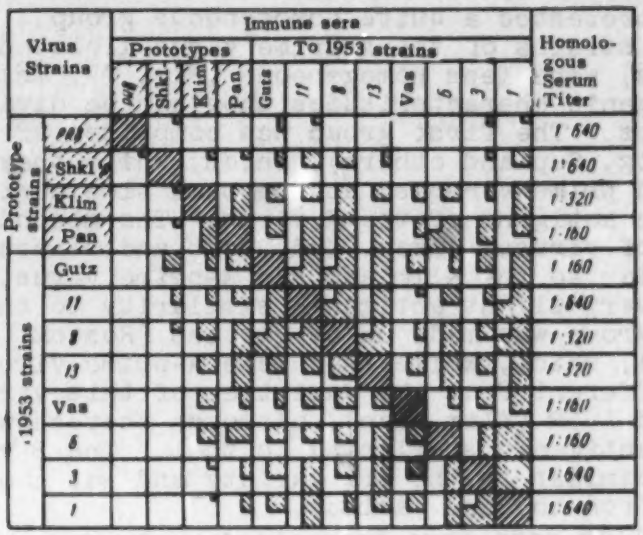

Fig. 78. Antigenic Structure of Influenza A-Prime Virus Strains Isolated in 1953. (Zakstel'skaya, 1954). The key is the same as for Fig. 76.

Table 41

Serological Characteristics of Influenza Virus Strains Isolated in December 1953

Rat antiserum against viruses \ Strain		A_1			A		Freshly isolated				
		YeM	S5	LP	PR8	Shkl-yaver	Bk	SPZ	BP	TN	Vorob.
A_1	Yem-49	320	32)	160	0	0	160	160	40	640	40
	S5-52	20	320	320	0	0	20	320	0	20.	80
	LP-52	0	320	640	0	0	640	320	320	160	0
A	PR8	0	0	0	160	20	10	0	0	0	0
	Shklyaver	0	0	0	80	640	20	0	0	40	0

The rules and regulations which we noted have also been observed in subsequent years by many authors who have come to similar conclusions.

Smorodintsev (1948) pointed out that the plasticity of the influenza virus leads to the fact that it changes its antigenic structure during the course of the epidemic process, Solov'yev and Marennikova (1952) have developed simultaneously with us very similar views as to the variation of the influenza virus in the epidemic process, basing their views on experimental material.

In the work of Smorodintsev, Kryachko and others (1953) differences have been noted distinctly in antigenic structure not only between the A and A-prime viruses but also between the strains of A-prime viruses isolated in Leningrad in 1949 and 1952 (Fig. 79).

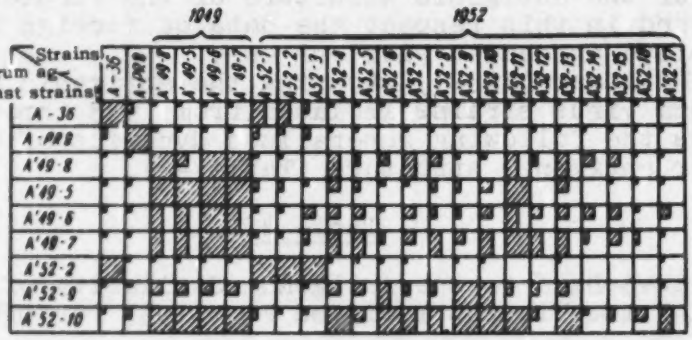

Fig. 79. Antigenic Structure of Strains Isolated in Leningrad in 1949-1952 (Luzyanina, 1953).

Key is the same as for Fig. 76.

A considerable number of similar observations has been published in recent years in the foreign literature.

Hilleman, Mason and Rogers (1950) have noted that the strains of A-prime virus isolated in the United States at the beginning of 1950, although they were similar to strains isolated in 1947, were still somewhat different from them in their antigenic structure.

Smith (1952) has noted that from 1933 through 1935 type WS was most frequently encountered among the A viruses; from 1935 through 1947, the majority were type PR8; and beginning with 1946 A-prime viruses appeared, whereby the 1949 strains already showed differences from the 1947 strains,

and the 1952 strains were markedly different from the 1947 strains.

Hirst (1952) studied 61 strains of influenza A-A-prime viruses and divided them into seven groups in which the definite serologic types (antigenic varieties) are characterized by a definite chronological sequence of their occurrence.

Magill and Jotz (1952) studied the antigenic structure of 100 strains of the influenza virus isolated in the past 18 years, and he separated out five antigenic subgroups among the A-A-prime viruses and four subgroups among the B viruses. A chronological table of isolation of the strains of the antigenic subgroups indicated has been presented in the work of the authors.

Here, there is no need to recriticize the serological classifications of influenza viruses proposed by Smith, Magill and Jotz, since this has already been done in another place (section 27). The very distinct tendency toward evolution of the antigenic structure of the viruses should be noted, and in this respect the data of foreign authors are in complete agreement with our own.

Hilleman (1952), studying the antigenic structure of influenza virus strains isolated from 1933 through 1950, presents the following generalized dynamics of the changes in their antigenic structure (Table 42).

Table 42

Generalized Data on the Antigenic Similarity of Influenza A-A-Prime Viruses Isolated From 1933 Through 1950

Sera	Antigens (strains)					
	1933	1943	1947	1948	1949	1950
1933 (WS)	++++	+	+	0	0	0
1943 (PR8)	++	++++	+	+	0	0
1947 (FM1)	+	+	++++	+++	+	+
1948 (Wlg)	+	+	++++	+++	++	+
1949 (Stl-49)	0	0	++++	++++	++++	++++
1950 (FW1-50)	0	0	++++	++++	++++	++++

Key. ////, ///, //, /, zero represent varying degrees of serological reactions.

It is fitting to present the data of Hilleman and Horsfall (1952) here on the study of the antigenic structure of the A-A-prime viruses isolated in various countries from 1933 through 1951. For the purpose of graphic presentation, the results of the investigations of these authors have been presented graphically according to the Gorbunova system (Fig. 80).

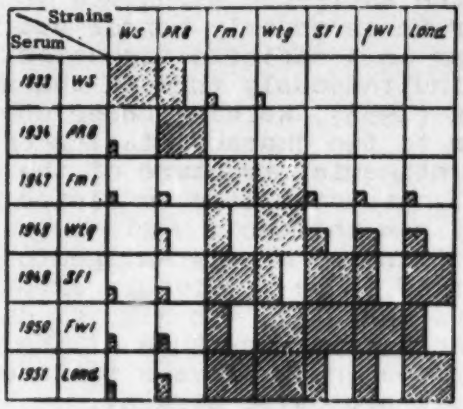

Fig. 80. Antigenic Structure of A and A-prime Viruses Isolated in 1933-1951 (Hilleman and Horsfall, 1952). The key is the same as for Fig. 76.

Tamm, Kilbourne and Horsfall (1950) compared the antigenic structures of B viruses isolated in New York in 1945 and 1950, and showed that the 1950 strains were notably different in the antigenic structure from the Lee strain and the 1945 strains in the neutralization and hemagglutination-inhibition reactions.

In conclusion, it is expedient to present data concerning the antigenic structure of influenza virus strains isolated in recent years simultaneously in various countries the study of which was organized by the World Health Organization in Geneva and by the so-called "Influenza Center" in London. During the influenza epidemic in Europe in the winter of 1948-1949, 86 strains of A-prime viruses were obtained from various governments: they were all identical in their antigenic structure (Chu and others, 1950). In 1950-1951, 96 strains of A-prime viruses were studied which had been obtained from various countries: all these strains belonged to the so-called "Scandinavian and Liverpool sub-

types" and in the antigenic respect they were slightly different from the 1947 and 1949 strains (Isaacs, Gledhill and Andrewes, 1952).

Strains of the B virus isolated in 1952 on four continents were studied by Bozzo (1952) and Hennessen (1952); they were all identical or similar to one another in their antigenic structure, similar to the crawley and different from the Lee strain.

The material presented shows quite convincingly that the changes in the antigenic structure of influenza viruses do not occur in an orderly manner but rather progress from year to year in a definite direction, whereby this evolution occurs simultaneously through the world.

Gorbunova (1953), working under our direction, directed attention to two characteristics of progressive changes in the antigenic structure of the influenza viruses.

The first characteristic consisted of the fact that the formation of new antigenic varieties occurs first by means of the building up of new antigenic groupings with the preservation of those previously existing, which latter are lost only subsequently. In Fig. 81 data are presented concerning the antigenic structure of the main (prototype) strains of A-A-prime and B viruses isolated in the USSR (including the Lee prototype strain).

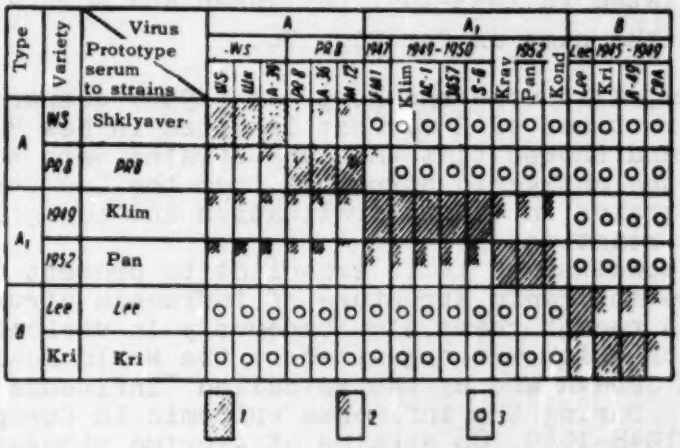

Fig. 81. Schematic Representation of Antigenic Structure of Influenza Virus in Accordance with the Result of the Hemagglutination-Inhibition Reaction of Various Strains of Influenza, A, A-Prime and B

Viruses With Type-Specific Standard Antisera.

1 -- serum titer with homologous virus or with the heterologous virus equal to one; 2 -- fractions of the titer (1/2, 1/4, 1/8); 3 -- titer with heterologous virus equal to less than 1/32 of the titer of the given serum (Gorbunova, 1953).

From Fig. 81 it is seen that the strains isolated in lated years (belonging to antigenic varieties which arose later) contain new antigens which distinguish them from the preceding strains, and at the same time they preserve the antigens of the latter to a considerable degree, losing them only gradually. This characteristic is most clearly seen through a comparison of the degree of similarity of the three A-prime influenza varieties of viruses (Klim, Pan and Rostok) with the A viruses (WS and PR8): whereas strains of A-prime virus of the 1949 vintage (Klim type) still have a definite antigenic similarity to the A viruses, the A-prime virus strains of the 1953 origin (Rostok type) have almost completely lost it. If they did not have antigens in common with the earlier varieties of A-prime viruses (Pan, Klim) they would have had to be eliminated from the A-A-prime group and classified as a completely new serological type (Ritova, 1953; Maksianovich and Il'yina, 1953).

This characteristic of change in the antigenic apparatus during the course of evolution of the influenza viruses was noted by Jensen and Francis (1953) who, by using the method of antibody adsorption, found 18 antigenic components in the A-A-prime viruses isolated from 1931 through 1953. They also showed that the formation of new antigenic varieties occurs by the means of the acquisition of new antigenic components and that only afterwards did they lose the old components (Fig. 82).

The second feature of changes in the antigenic structure of influenza viruses which has been expressed by way of a hypothesis by Zhdanov (1949, 1950) and experimentally proved by Gorbunova (1952), consists of the fact that changes in the antigenic structure, accumulating gradually, periodically lead to deep-seated qualitative changes -- the formation of a new biological type as a variety of the influenza virus. This situation has been analyzed in detail by Gorbunova through the example of the formation of the A-prime virus.

Studying the influenza A viruses isolated from 1933 through 1948 and the A-prime viruses isolated from 1947 through 1952, Gorbunova directed attention to the fact that these two groups of viruses, which according to their conglomeration of biological properties should be considered different biological types or varieties, not only have an-

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tigens in common (the extreme representatives of them -- the A viruses isolated in 1933-1934 and the A-prime viruses isolated in 1953 -- have practically no antigens in common) but are also associated with one another through intermediate strains. These strains were isolated during the period from 1941 through 1949 in different countries: in the United States the TW strains were isolated in 1941 and the Johnson strain was isolated in 1943 (Salk and Francis, 1946); in the USSR, the A-3 and A-9 strains were isolated in 1949 (Smorodintsev, 1950).

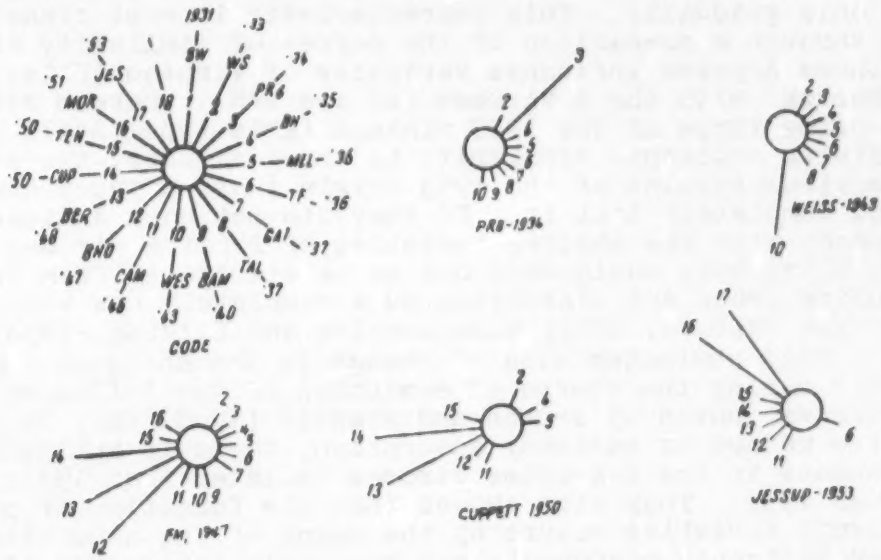

Fig. 82. Antigenic Structure of Influenza A and A-Prime Viruses (Jensen and Francis, 1953).
Each ray represents an antigenic component which has been given a number and a literal designation according to the name of the strain and the year of its isolation.

In the study of the antigenic structure of the TW strain, it was shown to have a mutual antigenic relationship with the PR8 virus; the PR8 serum inhibited the hemagglutination of this strain up to 0.07 of its titer with the homologous serum; the TW serum inhibited hemagglutination of the PR8 strain up to 0.06 of its titer. The sera of a number of A-prime strains (Chizh, Gavr, Bel) inhibited the hemagglutination of this strain up to 0.06-0.05 of its ti-

ter, but the serum of the TW strain did not react with a single A-prime strain (Table 43). On the basis of these data the TW strain was classified among the transitional strains from the A PR8 viruses to the A-prime viruses. Similar relationships were obtained through a comparative study of the Johnson strain with the A (PR8) and A-prime viruses.

Table 43

Antigenic Structure of TW Strain (United States of America, 1941) According to the Data of Cross Hemagglutination-Inhibition Reactions

Serum to strain	Virus strains										
	WS	PR8	A36	Chizh	Gavr	S6	Klim	A3	A9	AS1	A:83
WS	1	0,06	0,13	0	0	0	0	0	0	0	0
PR8	0,07	1	1	0	0	0	0	0,04	0,06	0	0
A36	0,06	1	1	0	0	0	0	0,03	0,03	0	0
Chizh	0,05	0,2	0,2	1	1	1	1	0,3	0,25	1	1
Gavr	0,25	0,15	0,1	0,75	1	1	1	0,5	0,5	0,75	0,9
C3	0,13	0,13	0,13	0,75	0,75	1	0,6	0,5	0,4	1	1
Bel	0,06	0,2	0,13	1	0,0	0,75	1	0,25	0,3	0,8	1
S6	0	0,04	0,02	1	1	1	1	0,2	0,3	1	1
Klim	0	0,06	0,03	0,3	1	0,6	1	0,4	0,3	1	1
Lshcho	0	0,06	0,03	1	0,75	1	1	0,3	0,25	1	1
Sdb	0	0	0	0,5	1	0,75	1	0,5	0,3	0,5	1

Key. The decimal represents the relationship of the heterologous serum titer to its homologous titer; unity represents the homologous serum titer or a coincidence of the heterologous titer with the homologous titer; zero represents the relationship of the titers which constitute a fraction less than 0.01 or a complete absence of hemagglutination inhibition of the heterologous virus in a serum dilution of 1:10.

In the study of the antigenic structure of A3 and A9 strains it was made clear that they are similar to the A WS viruses. The degree of their antigenic similarity to

the prototype strains of this variety (the Shklyaver and A39 strains) was expressed by the figures 0.4-0.6 (with respect to A3 and A9 sera) and 0.3-0.4 (with respect to Shklyaver and A39 sera). At the same time, they showed a quite pronounced, even if unilateral, relationship to the Chizh, Gavr and Bel strains of A-prime viruses: the figures for the antigenic relationship of the A-prime strains mentioned with respect to the sera were within limits of 0.3-0.5, but the corresponding figures for the A3 and A9 strains were no greater than 0.03-0.06.

Strains of this kind, intermediate between A and A-prime viruses, were isolated during the period 1941-1945 in other countries also (Sweden, Australia).

We considered it possible to look upon these findings as an expression of species-forming variability. During the period 1941-1945 changes in the antigenic structure occurred in the viruses circulating among the population which were completed in 1945-1947 with the formation of a new species -- the influenza A-prime virus. This virus was formed on the basis of the A virus, whereby the source of its formation could have been both principal varieties -- PR8 and WS.

It goes without saying that the appearance of new virus strains does not signify the immediate disappearance of the old strains. The latter continue to be isolated from influenza patients, although much less often than before the appearance of the new strain. The observations of Smorodintsev and his co-workers (Smorodintsev, Vlastelitsa and others, 1950; Smorodintsev, Luzyanina and others, 1952; Smorodintsev, Klyachko and others, Luzyanina, 1953) as well as observations made in our laboratory (Gorbunova, 1951, 1953) are evidence of this. Cases of isolation of A virus similar to the old varieties (WS, PR8) in children, elderly persons, inhabitants of remote islands (Hilleman, 1951; van Rooyen and others, 1949) (Nagler and co-authors, 1949, and others) also attest to this.

It seems interesting now to analyze the problem of the factors and conditions leading to variability in the antigenic structure of influenza viruses. Before expressing any considerations on this problem it is necessary to analyze the factual material which has accumulated to date.

31. Phasic States of the Influenza Viruses

At the beginning of the 1940's a number of authors expressed the idea of the existence of phasic states of the influenza viruses with which certain characteristics of influenza epidemiology were associated, specifically that

from time to time epidemics of it occurred, after which long intervals followed in which the influenza morbidity rate was low.

Burnet and Clark (1942) believe that the influenza virus undergoes periodic fluctuations in its virulence which correspond to variations in the intensity of the epidemic process. Andrewes (1942) expressed the idea that the influenza virus undergoes a whole cycle of transformations in its antigenic structure during the course of the epidemic process, beginning with the avirulent stage of unified antigens and ending with a highly virulent stage enriched with antigenic groupings; the first stage of the virus is characteristic of sporadic cases of influenza; the latter, of epidemics and pandemics. Since these hypotheses were of a purely speculative nature and were not confirmed by facts they did not receive recognition among virologists.

Similar views have also been expressed in the Soviet literature. Zhilkina (1948, 1952) and Sakharov (1952) indicated the existence of influenza virus in an antigenically altered form during the interepidemic period, and a widespread healthy carriage of the virus was associated with it. Zhilkina based her conclusions on the use of imperfect investigation methods, which were justifiably criticized by Smorodintsev (1951).

Recently, this conception has gained new strength in the foreign literature in connection with the finding of certain facts concerning the phasic states of the influenza virus. The essence of these facts consists of the following.

Certain authors who have worked on the study of the antigenic structure of influenza virus strains by the method of the hemagglutination-inhibition reaction have noted cases of a more pronounced effect of the sera on the heterologous virus strains than on the homologous strains. Mulder and others (1949) grouped the A-prime strains into three types (P, Q, R) in accordance with behavior characteristics of the viruses in the hemagglutination-inhibition reaction. The strains which react with homologous serum in its highest titer and with heterologous sera of the same serological group to a considerably lesser degree belong to the first type, P. Strains which react weakly with all sera including the homologous serum belong to the second type, Q. Strains which enter into the hemagglutination-inhibition reaction with all sera of a given serological group on a par with the homologous serum belong to the third group, R. Table 44, which is a reproduction from Mulder's work, reflects the manner of reaction of the P, Q, and R strains in cross hemagglutination-inhibition reactions with their antisera in a general form.

Table 44

Diagram of Antigenic Structure Within the Same Subgroup of Strains of the A-Prime Type (Mulder, 1949)

Serum against strains	Strains		
	P	Q	R
P	++++	+	++++
Q	+	+	++++
R	+	+	++++

Key. Plus low titer; /// high titer.

The R strains, according to Mulder, are ordinary A strains (PR8) which react well with the homologous and heterologous sera of the same serological type. The Q strains can be used for determining their structure only in the event Q serum and R strains are used in the hemagglutination-inhibition reaction. The P strains are not very well suited for work on the identification of new viruses, since they react weakly with the heterologous sera of related serological groups.

The division of newly isolated strains into P, Q and R types were used by Isaacs and Andrewes (1951) as well as by Isaacs (1951, 1953) in the study of the 1950-1951 epidemic according to material from the laboratory of countries of the Eastern Hemisphere unified by the World Influenza Center in London. Isaacs and Andrewes used the Mulder system in the study of the antigenic structure of the A-prime strains. They divided all the strains isolated during the 1950-1951 epidemic into two main types -- Scandinavian and Liverpool. The Scandinavian type possessed the properties of the Q strains, according to the Mulder system; the Liverpool type corresponded to type P. Strains of the Scandinavian type were isolated in areas of Northern Europe (Sweden, Denmark, Belgium, Germany, Finland, Iceland); strains of the Liverpool type were sent in from the laboratories of the countries along the Mediterranean Sea (France, Spain, Greece, Turkey, Israel). The strains of both types were isolated in England, Ireland, Italy, South Africa, and Australia.

The main idea in the works of Isaacs and Andrewes is the fact that strains of the Scandinavian type are cap-

able of withstanding the specific effect of virus-neutralizing antibodies. At the same time, strains of the Liverpool type possess a higher degree of avidity for the specific serum antibodies.

The epidemiological significance of types P, Q, and R, in the opinion of these authors, consists in the fact that they are phasic states of the influenza virus. On behalf of the latter hypothesis the authors advance the fact of the existence of both types of virus simultaneously in many countries. Since the antibodies are a protective mechanism against influenzal infection and the Q viruses are weakly neutralized by antisera, it is natural that the virus in the Q phase will be better adapted to the organism than viruses in the P and R phases.

Afterwards, there were several further attempts to associate the P and Q variations in the influenza A-prime viruses with the characteristics of the epidemiology of the diseases. In one of their recent works on this subject Isaccs and Archetti (1954) investigated 400 strains of A-prime virus isolated in various countries from 1950 through 1954. Thereby, strains were isolated in various countries which were chiefly of the Scandinavian type or of the Liverpool type or both. The authors attempted to show that the latter crowd out the former; however, the material which they present is extremely unconvincing.

Andrewes (1951) recalled his 1942 works in which the existence of a "basic" virus was postulated devoid of antigenic properties (which is completely improbable) and virulence. In the journal "Lancet" an editorial was published under a characteristic heading, "Masks of a Virus," in which mention is made of the influenza virus' going "underground". The work mentioned above by Isaacs and Andrewes has a title which may be translated as "pathways of influenza spread". It is hardly necessary to prove the inexpediency of such a method of solving scientific problems.

In reviewing our experimental material dealing with the results of cross hemagglutination-inhibition reactions in the light of the assumed existence of the Liverpool and Scandinavian types of virus we noted that the behavior of certain of our strains can be compared with the state corresponding to the P, Q, and R forms.

Here we should like to present examples of different virus behavior in the hemagglutination-inhibition reaction with specific antisera (Table 45).

In Table 45, the hemagglutination-inhibition reaction of three allantoic cultures of the same B virus, the Kri strain, are shown. The cultures represent similar virus passages in embryos. The reaction was performed simul-

taneously with three sera against the PR8, Lee and Kri strains.

Table 45

Various Allantoic Cultures of the Kri (B) Strain in Accordance with P-Q-R-Forms of Variations

Serum against strains	Allantoic Cultures of Kri Virus		
	P	Q	R
	7Ya 1m 38Ya	7Ya 1m 37Ya	7Ya 1m 41Ya
PR8 6/XII 1950	0	0	0
Lee 6/XII 1950	20	40	160
Kri 6/XII 1950	320	80	160

Key. The figures represent the serum titers in the hemagglutination-inhibition reaction; zero represents the absence of a hemagglutination-inhibition reaction. 7ya 1m and 38ya signify that the virus has had seven passages in chicken eggs; one, in mice; and 38, in eggs again.

It was shown that the three virus cultures may be regarded as different phasic states of the strain.

In addition to these three Kri cultures two allantoic fluid cultures of the same strain and five different Lee cultures were also used in the experiment. The aim of the experiment consisted in checking the specificity of Lee serum. Judging by the results of these experiments the Lee serum should be considered specific for B strains since the other seven cultures mentioned (two Kri and five Lee) behaved typically, that is, the homologous serum titers with these cultures were the highest and the heterologous titers were correspondingly lower.

For the purpose of explaining the phenomenon of the different neutralizing effects of the same Kri serum on the homologous Kri strain, represented by the three separate allantoic fluids, a supposition should bemade as to the presence of individual properties of the various virus cultures, which perhaps depend on the physio-chemical state of the allantoic fluid.

Control experiments with Lee and PR8 sera and their homologous strains indicate the fact that the antigenic difference depends on the individual nature of the cultures rather than on the characteristics of the sera.

The absence of inheritance of the phasic state is proved by the fact that the three Kri cultures in Table 45 come from one another in accordance with the manner in which they are numbered. This experiment once again shows the expediency of preparing the antigen for the hemagglutination-inhibition reaction by means of mixing several allantoic fluids.

Hirst in 1943 pointed out the individual avidity of each of the various allantoic cultures of influenza virus reacting with specific antisera, suggesting that a correction be made for the avidity factor in the hemagglutination-inhibition reaction and reducing the absence of reciprocity of the cross reactions to the nonspecific individuality of the allantoic cultures of influenza virus.

It seems to us that Hirst was right in pointing out the particular avidity of certain allantoic fluids infected with virus for entering into a hemagglutination-inhibition reaction in reacting with specific immune serum, but he was not right in his interpretation of the absence of reciprocity of the cross reactions where they are checked through a number of repeated experiments with different types of allantoic virus cultures.

As an example of the study of serological types on the basis of the distinct behavior of the strains in the hemagglutination-inhibition reaction we may also refer to experiments with the Swedish virus, A-Sweden 6/50. According to the data of Isaacs, this strain is a representative of the Scandinavian serological type, that is, the Q type according to Mulder.

We have three types of S6 (A-Sweden 6/50) antisera at our disposal which were prepared in our laboratory by Fadeyeva. These were sera: 1) against the S6ya strain line which had been passaged continuously in chick embryos; 2) against the S6m strain line, that is the strain variant adapted to mice; 3) the S6h strain line, that is the strain variant adapted to tissue of a human embryo (Table 46).

An analysis of the results of the reaction with sera and the A-Sweden 6/50 virus shows the absence of any kind of distortions in the behavior of the three lines of this strain.

In all the three examples the reaction between the homologous (in the sense of adaptation) strain and its antiserum is the best one (the homologous titers are the highest).

Therefore, the A-Sweden 6/50 strain in our experiments did not show any deviations from the behavior of other A-prime strains in the hemagglutination-inhibition reaction. At the same time, through the experiments of

Fadeyeva (1952) on the adaptation of this strain to human lung tissue in vitro and on the application of the tissue variant in experiments on volunteers the prolonged survival (three to five days) of the virus on the mucous membrane of the human nasopharynx was established.

Table 46

Serological Analysis of S6 Strain Variants in the Cross Hemagglutination-Inhibition Reaction

Rat sera against strains	Strain S6 variants			A-prime strains (March 1949)	
	S 6Ya	S6m	S6h	Klim	Lahcho
S6Ya	80	40(1/2)	80(1)	40(1/2)	40(1/2)
S6m	160(1/4)	640(1)	320(1/2)	320(1/2)	320(1/1)
S6h	320(1/2)	160(1)	640(1)	640(1)	320(1/2)

Key. The whole numbers represent the absolute serum titer; the fraction represents the ratio of the heterologous serum titer to its homologous titer.

The survival of the S6 strain may be considered from the point of view of its belonging to the Scandinavian group, the distinguishing characteristic of which is a capacity to adapt itself to growing even in the presence of specific antiserum. However, the interpretation of the success of Fadeyeva's experiments is based on another premise. We recognize the justifiability of Fadeyeva's conclusions to the effect that the survival of the virus should be considered a preliminary adaptation to the human tissue protein in in vitro passages, which has been confirmed by the recent experiments of Zakstel'skaya and Ritova (1953).

Similar data have been obtained by Solov'yev and Marennikova (1951) with the YeM-prime and YeM-double-prime strains of influenza A-prime virus, of which the former was adapted to chick embryos and the latter, to mice.

An analysis of the interrelationships between the YeM-prime and the YeM-double-prime strains and their antisera makes it possible to establish the fact that the YeM-prime virus elicits a low degree of activity of the homologous YeM-prime and that the other A-prime strains can be arranged in the following series according to the hemagglutination-inhibition reaction with YeM-prime serum:

1. The Gav, Bel, Klim strains show antihemagglutinating properties of the YeM-prime serum just as poorly as the YeM-prime strain does, in the reaction with which the serum titer remains equal to 1:40.

2. The Chizh, Yaks, S6 strains, in the reaction with which a higher YeM-prime serum activity is found, since its titer (1:80) is twice as high as the homologous titer (1:40).

3. The Sdb, Lshcho, S3 strains, by means of which it is possible to establish an antihemagglutinating activity of YeM-prime serum in a dilution of 1:160, that is, exceeding its homologous titer by four times.

Therefore, an inverse relationship may be noted between the ratios for the heterologous and homologous serum titers against the strains mentioned with the YeM-prime virus and of YeM-prime serum with the same virus strains. These figured data permit us to draw the conclusion that there is a slight difference in antigenic structure between the YeM-prime strain and the Sdb, S3, and YeM-double-prime strains.

On the other hand, there is an indubitable similarity between the YeM-prime strain and its YeM-double-prime variant, which is shown, as is mentionedabove, in the hemagglutination-inhibition reaction of the YeM-double-prime virus by YeM-prime serum. In Figs. 83 and 84 the unusual nature of the hemagglutination-inhibition reaction with the participation of YeM-prime serum is demonstrated compared with the interrelationships usually observed between strains.

Therefore, the YeM-prime strain may be regarded as an example of the Q type of Mulder and, therefore, of the Scandinavian type of viruses. The more active reaction between the serum and the heterologous R strains but not with the P strains constitutes a similarity to the Q type.

The mechanism of this reaction is unclear, since it shows an undoubted activity of the YeM-prime serum and the YeM-prime virus with respect to entering into a hemagglutination-inhibition reaction with heterologous antigens and sera. Thereby, a quite pronounced specificity of both the YeM-prime strain and its antiserum is shown which corresponds to the A-prime type. The weak capacity of the YeM-prime strain for entering into a hemagglutination-inhibition reaction with homologous antiserum is striking, and the avidity of the mouse-adapted variant for the same original serum against the YeM-prime strain is unusual, despite the essential difference in the antigenic structure of the YeM-double-prime variant, which is expressed in a neutralization of the YeM-prime strain by serum against the YeM-double-prime strain to the extent of only one-eighth of its titer. However, this difference may be interpreted

also from the point of view of the lack of capacity of the YeM-prime strain to react with the YeM double-prime antiserum which is identical in its antigenic structure. This absence of avidity should be referred to the account of the virus and may be explained tentatively by the instability of the virus reaction with antiserum, the relative ease of dissociation of the neutral complex into its constituent elements, for example, after the addition of a suspension of erythrocytes.

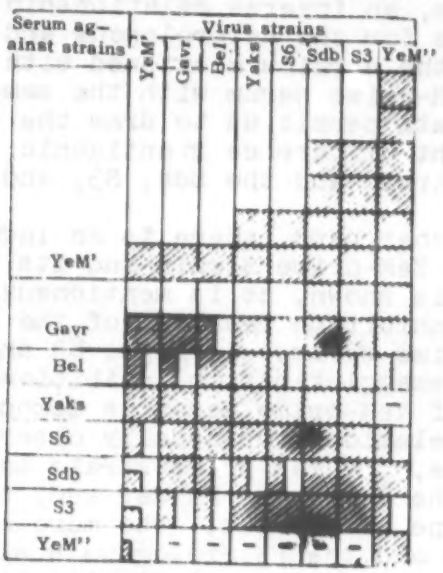

Fig. 83. Distinctive Relationships Between the Heterologous and Homologous Serum Titers Against YeM-Prime Strain Depending on the Use of Different Strains in the Hemagglutination-Inhibition Reaction.

The whole hatched squares represent the homologous serum titer or the heterologous serum titer equal to the homologous serum titer; the squares which are half hatched or one-quarter hatched represent the serum titer which is equal to 1/2 or 1/4 of its homologous titer; two or more hatched squares represent a heterologous titer which is two or more times the homologous titer; the minus sign represents the absence of corresponding experiments (Gorbunova, 1953).

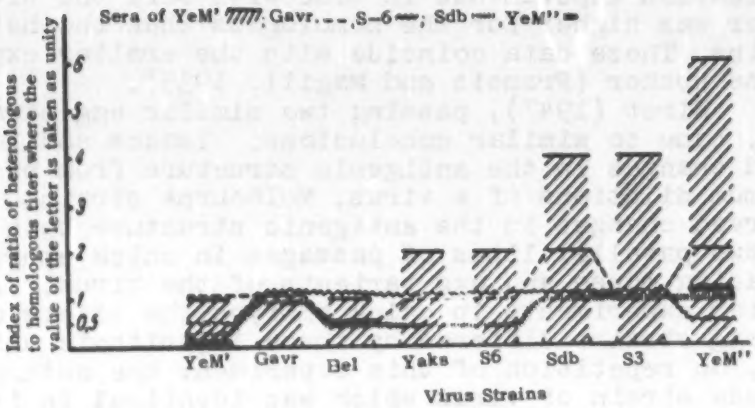

Fig. 84. The Relationships Between the Heterologous Serum Titers Against the YeM-Prime Strain and its Homologous Titer in Comparison with the Same Interrelationships of Other A-Prime Sera (Gavr, S6, Sdb, YeM'').

The value of the indices of the heterologous serum titer ratios to the homologous titers is represented in the form of a curve for each serum separately (see the designations of each serum); on the ordinate axis, the value of the ratios is shown; on the abscissa axis, the names of the virus strains are noted which participate in the hemagglutination-inhibition reaction with the sera mentioned (Gorbunova, 1953).

32. Factors Influencing the Antigenic Structure of the Influenza Virus

The Antigenic Structure and the Biological Type of the Host. Study of the effect of cultivation of influenza viruses in the bodies of various species of animals or in tissue cultures of them has made it possible to accumulate data which are quite contradictory.

Francis (1947) compared two lines of the same influenza virus strain (PR8), of which one was passaged for a long time in mice; the other, in chick embryos. He came to the conclusion that the strain variants show certain serological differences from one another.

In experiments with cross immunization of mice a mouse-adapted strain was found to be 100 times more immuno-

genic than a strain passed through chick embryos. In neutralization experiments in mide with sera the titer of the latter was higher for the homologous than the heterologous strains. These data coincide with the eralier experiments of the author (Francis and Magill, 1935).

Hirst (1947), passing two similar egg strains in mice, came to similar conclusions. Isaacs and Edney (1950) found changes in the antigenic structure from passages of maximum dilutions of A virus, Melbourne strain. Sugg (1949) observed changes in the antigenic structure both in comparing two parallel lines of passages in chick embryos and in comparing mouse and egg variants of the virus. In neutralization experiments in chick embryos the titers of the mouse and egg strains differed by three logarithmic units. However, on repetition of this experiment the author obtained a mouse strain of virus which was identical in its antigenic structure to the egg strain, although it differed with respect to its virulence for mice.

Smith (1952) notes a greater degree of plasticity of the influenza virus as a result of which the adaptation of its egg strain to the bodies of mice leads to changes in the antigenic structure. Solov'yev, Marennikova and Gutman (1953) compared the allantoic and mouse variant of the influenza A-prime YeM virus strain and came to the conclusion that there is a certain change in the antigenic structure of the mouse strain compared with the allantoic strain.

In these experiments, however, the question was not so much that of antigenic structure as of the different strength of the antigenic stimulation which was produced by the corresponding strains, that is, of immunogenic properties.

At the same time, the many years of practice in the work of virological laboratories indicates a pronounced stability of the antigenic structure of influenza viruses during the course of their being passaged in the bodies of various animals (mice, ferrets, chick embryos) and in tissue cultures. Certain research which has been especially performed also constitutes evidence of this.

Shen and Vanag (1948, 1954) established the fact that during the course of adaptation to tumor tissue many biological properties of the influenza virus are changed but not the antigenic structure. Baydakova and Zil'ber (1948, 1949) also observed changes in the biological properties of the influenza viruses during the course of adaptation to the bodies of mice; however, the antigenic structure was not involved thereby. Blyumberg (1949) adapted the influenza virus to the bodies of guinea pigs. The virus, which was very much changed by this procedure, pres-

erved its previous antigenic structure.

In the experiments of Luzyanina (1952, 1953) passages of influenza viruses through the bodies of white rats did not reflect on their antigenic structures, as is seen from the protocols of her investigations. In similar experiments of Peterson (1954) the virus, which had lost its pathogenic properties, maintained completely its antigenic structure in the complement-fixation reaction.

Magill and Francis (1936) also have noted that the virus strains which had been passaged previously in ferrets and mice maintained their antigenic structure after many scores of passages in tissue cultures of chick embryos. Shope and Francis (1936) did not observe any changes in the antigenic structure of influenza viruses after passages of them in young pigs.

Tamm, Ginsberg and Horsfall (1952) made a special study of the problem of whether the antigenic structure of influenza viruses changes when they are isolated and subsequently passaged in chick embryos. Using a sensitive method of cross neutralization in eggs, the authors did not find any notable differences in the antigenic structure of strains which had undergone different numbers of passages in chick embryos.

Since this problem is of importance in principle for understanding the rules and regulations of virus variability, special investigations were performed in our laboratory which had the aim of clarifying the question of whether or not the antigenic structure of influenza viruses is changed during the course of adaptation to the bodies of various hosts and tissue cultures.

Fadeyeva (1952, 1953) made a serological analysis of the influenza A (Shklyaver) and A-prime (S6) virus strains which had undergone a series of passages in chick embryos, mice, and tissue cultures of mice and man, preparing rat immune sera for each variant of the strain. In Fig. 85 the summarized data on the results of the serological research done by the author are presented.

The author comes to the conclusion that even prolonged virus passages in developing chick embryos or through the bodies of normal adult mice as well as in tissue cultures prepared from embryonic mouse or human tissues did not produce any kind of apparent changes in the antigenic structure which could be detected by cross hemagglutination reaction.

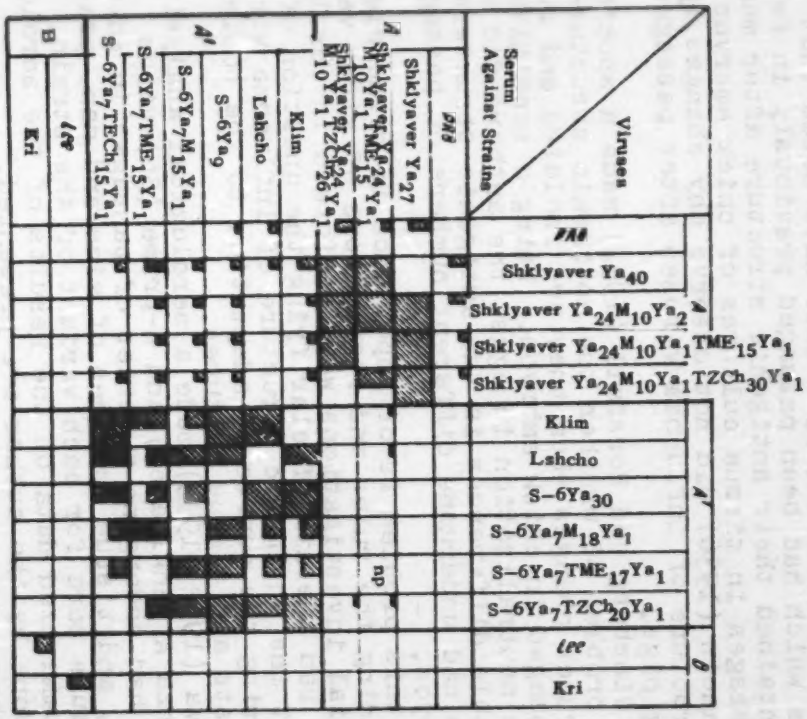

Fig. 85. Serological Analysis of Strain Variants of Influenza Viruses (Fadeyeva, 1952).

The area of the hatched square is proportional to the degree of antigenic similarity; np -- experiment not performed.

Gorbunova (1953) investigated the antigenic structure of the same influenza virus strains which had been passaged in chick embryos and mice, and did not find any changes in the antigenic structure on comparison of the corresponding strain variants. The following observations attest to the stability of the antigenic structure of the influenza virus during the course of its being passed through laboratory animals. The well-known Lee strain of the influenza B virus was obtained from the United Stats at the beginning of the 1940's and since that time has been extensively utilized in the laboratories of the USSR; specifically, it has undergone many passages in mice and chick embryos at the Institute of Virology of the Academy of Medical Sciences USSR. In 1952 the Lee strain was obtained again from abroad by the Institute of Virology of the Academy of Medical Sciences USSR. A comparison of the antigenic structure of both varients passaged in various places showed a complete identity of them.

Slepushkin (1954) compared the antigenic structure of the same influenza virus strains, A, A-prime and B, which had been passaged in chick embryos and human tissue cultures, and obtained data which were entirely similar to the data of Fadeyeva and Gorbunova.

Therefore, the results of the investigations in our laboratory show quite distinctly that during the course of adaptation to the bodies of animals in tissue cultures the antigenic structure of influenza viruses does not undergo any kind of essential changes detectable by serological methods of investigation. These data are in complete accordance with the everyday practical work of the virological laboratories and the results of investigations of many authors.

How can the observations of certain authors be explained who succeeded, as it were, in showing changes in the antigenic structure during the course of adaptation of influenza viruses to the bodies of various animals?

It seems to us that this could have depended on various causes. First, certain authors, in speaking about the antigenic structure of strain variants, actually have in mind the capacity of the virus for producing antigenic stimulation of various strengths, that is, its immunogenic properties. Secondly, in the experiments described above the avidity factor, of which we spoke above, was not taken into consideration, and therefore differences in the avidity of strain variants for antisera could have been taken as a difference in their antigenic structure.

As had already been mentioned, in the majority of works where this problem has been given special study it was not possible to detect any kind of essential changes

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in the antigenic structure of viruses multiplying in the bodies of various animals. In this connection, the works of Zeytlenok and Korshunova (1940) and Wang (1948), who made a special study of this problem, also deserve attention.

The first of the authors named studied strains obtained by direct infection of mice and chick embryos in neutralization experiments. They did not find any antigenic differences in the strains isolated in this manner.

Wang found considerable differences between the strain variants of the A-prime virus (Rhodes), of which one was passaged in mice and the other, in chick embryos. However, these differences did not apply to the antigenic structure, which was identical in both strain variants.

It seems expedient to dwell in greater detail on one work which pertains to the problem under analysis.

Isaacs and Edney (1950), passing influenza viruses in maximum dilutions, obtained variants which were different in their antigenic structure, in addition to other properties. Serum against the original Mel strain in the hemagglutination reaction suppressed the original strain in a titer of 1:1200 and the strain variant, in a titer of 1:700; the serum against the strain variant inhibited the strain variant in a titer of 1:3200, while theoriginal strain inhibited it in a titer of 1:150. In neutralization experiments on embryos the logarithms of the corresponding serum titers against the original strain were equal (calculating for the LD_{50}) to 6.3 and 3.3, while the sera gainst the strain variant were equal to six and 1.3.

It is not hard to see that the differences between these two strain variants does not go beyond the limits of the serologic type or even beyond the limits of the subtype of the given virus and correspond to variations usually observed for strains isolated during the same outbreak.

As seen from Fig. 85, certain variations of this kind, although less pronounced, were observed also by Fadeyeva.

Undoubtedly, any virus population is not entirely homogeneous, because no absolutely identical virus particles exist.

Through the method of selection (passages of maximum virus dilutions) small variants may be picked out of which the population consists, and therefore the data of Isaacs and Edney cannot be considered particularly surprising.

Approximately the same facts are observed in the comparison of mouse and egg variants of the influenza viruses (Gorbunova, 1953; Solov'yev and Marennikova, 1949, 1952; Marennikova, 1952).

In summarizing the results of the data presented we may come to the conclusion that adaptation to the bodies of various animals does not alter essentially the antigenic structure of the influenza viruses as determined by ordinary methods of investigation (hemagglutination-inhibition reaction, complement-fixation reaction, neutralization of the infectious properties by specific sera): the viruses continue to belong to the same serological type.

This, however, does not mean that the antigenic structure of influenza viruses is not changed at all in the adaptation to the bodies of hosts. As will be seen, changes occur in those properties of the viruses with which their immunological activity is associated. Undoubtedly, changes in the antigenic structure also occur thereby; however, these changes are finer and are detected by different investigation methods from ordinary serological analysis. Corresponding data will be presented in a special section.

The Influence of Physical and Chemical Factors on the Antigenic Structure of Influenza Viruses. Existing data show that the reaction of the medium (concentration of hydrogen ions), the presence in it of various elements, chemical agents (including antibiotics) do not lead to any changes in the antigenic structure of the viruses within the limits of those concentrations of the substances wherein the existence and multiplication of the virus are possible. The viruses maintain their antigenic structure also when cultivated at different temperatures. In the preceding chapter the effect of these factors on influenza viruses has been discussed in detail. In none of the works mentioned were any facts established as to a change in the antigenic structure of influenza viruses after the effect on them of various physical and chemical factors. Therefore, there is no need to return to these investigations.

Passages of Influenza Viruses in Mixed Cultures. Passages of influenza viruses in mixed cultures attracted attention after the publication of works by Burnet and his co-workers (Burnet, 1951; Burnet and Lind, 1951; Burnet and Edney, 1951), following which a considerable number of checking investigations appeared.

The essence of these investigations consists of the following. Burnet and Lind made intracerebral passages of a mixture of two strain variants of influenza WS viruses in mice: the neurotropic variant NWS and pneumotropic variant WSM. These strains were distinguished by a number of biological properties: the NWS strain possessed a pronounced neurotropism and weak enzymic activity (it did not destroy ovomucin) and a thermolabile hemagglutinin; the WSM strain did not possess any neurotropism, was enzymically

active and had a thermostable hemagglutinin (which withstood heating to 56° for 30 minutes). In intracerebral passages of the mixture of these viruses variants were isolated, along with the original neurotropic strain from the brains of mice, which possessed a combination of a number of properties of the original strains, including the NM neurotropic variant, but which were enzymically active with a thermostable hemagglutinin. The authors showed, using the method of passages of maximum virus dilutions in chick embryos, that the variants which they obtained do not constitute a mixture of viruses and that the acquired properties are stable.

In the next work Burnet and Edney, using the same method, obtained combined strain variants of the original viruses: the neurotropic A (NWS) strain and a pneumotropic virus, this time of another A strain (Mel). The strain variants also showed a combination of properties of the original strains.

Similar results were obtained by Appleby (1952), Burnet and coauthors (1953), Frazer (1953), Hirst and Gotlieb (1953). In these investigations combined strain variants were obtained with mixed infection of mice and chick embryos by neurotropic and pneumotropic strains of the influenza A, A-prime and swine influenza viruses.

Perry and Burnet (1953) obtained combined strain variants from the original B (Mel, Lee) viruses which were different in their serological properties and thermostability. Passages of mixed cultures were made by means of infecting mice intranasally (multiplication of the viruses in the lungs).

Finally, Gotlieb and Hirst (1954) obtained combined forms of A and B viruses (PR8 and Lee, Wsn and Lee strains) by means of cultivating mixed cultures in the allantoic cavity.

These data in all evidence attest to the existence of signs of vegetative hybridization of the influenza viruses. A critical analysis of possible interpretations of the facts obtained will be presented below. Here, we should like to note that in our laboratory similar facts have been observed by Gorbunova (1953, 1954), who investigated the behavior of A and A-prime viruses in mixed cultures, and afterwards by Ritova and Zakstel'skaya (1954) who obtained combined vaccine strains by means of passages of a mixture of viruses in cultures of human tissue.

Gorbunova (1953), in isolating the A-prime virus (Volk strain) from a patient, apparently ran up against contamination of it with the laboratory virus A (PR8) strain. The Volk strain isolated from the patient proved

to be a typical A-prime virus on serological examination and remained the same for five allantoic passages performed with implantations of concentrated material (allantoic fluid dilution of 10^{-2}); however, in the sixth passage it began to behave like a typical A (PR8) virus. By the method of passages of maximum dilutions of virus-containing fluid (10^{-6}-10^{-7}) it was possible to isolate pure lines of A and A-prime viruses along with which in certain infected embryos components were contained of both viruses. The antigenic characgeristics of the various types of allantoic fluid are presented in Fig. 86.

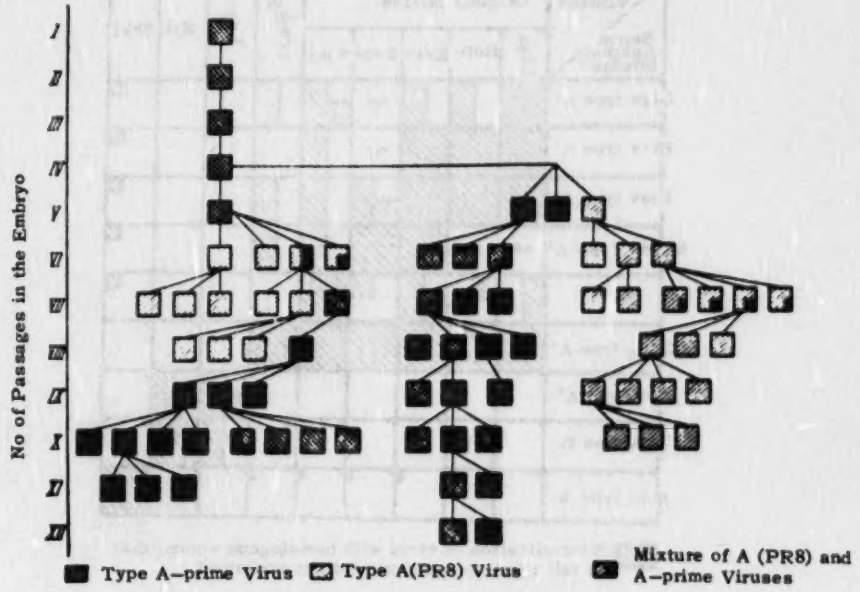

Fig. 86. Diagram of Isolation of A-prime and A Viruses from Mixed Volk Culture (Gorbunova, 1953).

Each egg is represented by a number and a square.

Mixed virus cultures have been used in our laboratory for the purpose of obtaining vaccine strains with a complete set of antigens (Ritova and Zakstel'skaya, 1954). With this aim in view, a mixture of five A-prime viruses (S6, Krav, Bayev, Liga and Botv), of which the first belonged to the

Klim variety and the other four, to the Pan variety, were kept in the desiccated form for eight months, and passaged through tissue cultures of a human embryo. As a result, the culture which did not seruvive well in the human nasopharynx, regained these properties after the third passage, and these were reinforced by the eight subsequent passages in tissue cultures. A study of the antigenic properties of the mixed culture (combined strain) showed that there is a complete set of antigens of the original five strains in it (Fig. 87).

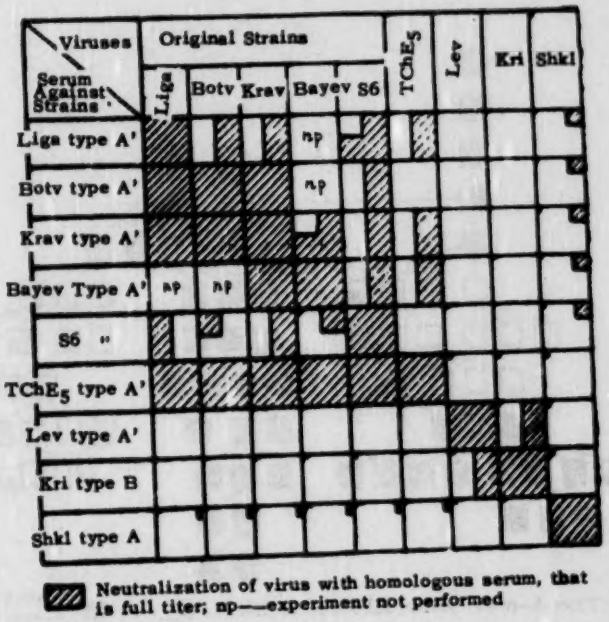

Fig. 87. Antigenic Structure of Original Strains and Combined $TChE_5$ Strain (Ritova and Zakstel'skaya, 1954).

Neutralization of virus with homologous serum, that is full titer; np -- experiment not performed.

Afterwards, combined vaccine strains were obtained from seven A-prime viruses and from two B viruses (Fadeyeva, Zakstel'skaya and Ritova, 1954).

Similar experiments according to the same method were

repeated by Smorodintsev (1954), who succeeded in obtaining a combined strain (mixed culture) from the original A and A-prime viruses as well as in introducing it into a vaccine.

Therefore, apart from its theoretical interest, the method of influenza virus passages in mixed cultures acquires definite practical significance for the purpose of obtaining vaccine strains with an enriched set of antigens.

The Variability of Influenza Viruses in the Immune Organism. The facts noted above of variation in the antigenic structure of influenza viruses in the epidemic process automatically directed the thoughts of investigators toward attempts at establishing experimentally the influence of a specific immunity or of its individual factors on the biological properties of viruses, primarily on their antigenic structure.

Investigations of this kind were undertaken by the Luzyanina (1950, 1952, 1953), Archetti and Horsfall (1950), Archetti (1952), Gavrilov (1953, 1954), Solov'yev and Gutman (1954).

Archetti and Horsfall passaged the influenza virus in chick embryos after a preliminary neutralization of it to the infectivity end point of heterologous serum, that is of serum against the same type (A) but a different variety of the virus. After a limited number of passages the authors found variants which were different in the antigenic respect from the original cultures. Thereby the variants created were, for the most part (with the exception of one), stable and preserved the altered antigenic structure after numerous passages in the absence of serum (Archetti). The authors consider the results which they obtained a result of mutations, believing that the serum is a selection factor in the "spontaneous mutations" of the virus: mutants neutralized by the serum are eliminated and mutants with an altered antigenic structure become predominant. The experiments of the authors leave many puzzling problems, both methodological and those pertaining to the treatment of the results obtained.

In Smorodintsev's laboratory Luzyanina repeatedly passaged the A (PR8) virus through the bodies of actively immunized mice. The immunization was carried out once with the intraperitoneal injection of formalinized PR8 virus, after which on the first to 10th day the mice were infected intranasally with the next virus passage. Owing to this, it was possible in a considerable number of mice to produce a fatal influenzal pneumonia (100-1000 MLD of virus were introduced) and to assure a continuous line of passages. During the course of the passages the author found a gradual change in the antigenic structure of the

virus revealed in experiments with cross hemagglutination-inhibition reactions and in neutralization experiments on mice. Changes in the antigenic structure were accompanied by an increase in the pathogenicity of the virus for animals.

In Table 47 data are presented of cross neutralization experiments with the original strain and with the strain which had come through about 60 passages in the bodies of immunized mice ("altered").

Table 47

Results of Neutralization Reaction of Original and "Altered" Strains by Corresponding Sera in Experiments on White Mice

Virus Dilution	Number of Mice Which Died After Infection					
	Original Strain and Original Serum	Original Strain and Altered Serum[1]	"Altered" Strain and Original Serum	"Altered" Strain and Altered Serum	Original Strain and Normal Serum	"Altered" Strain and Normal Serum
10^{-1}	4	4	4	3	4	4
10^{-2}	2	3	3	0	4	4
10^{-3}	0	3	2	0	4	4
10^{-4}	0	1	1	0	4	4
10^{-5}	0	0	0	0	4	4
10^{-6}	0	0	0	0	3	2
10^{-7}	0	0	0	0	0	0

[1] Serum against "altered" strain.

[1]Serum against "altered" strain.

In these experiments the original virus in a mixture with homologous serum caused the death of 50 percent of the mice in a dilution of 1:100, and with heterologous serum, in a dilution of 1:2000 with a virus titer of 1:2,138,000. The "altered" strain, when used for infection in a homologous mixture, produced the death of mice in a dilution of 1:2, and in a heterologous mixture, of 1:1000, in a virus titer of 1:100,000. Therefore, differences in the antigenic structure of these strains were revealed very clearly.

Detailed investigations on this subject were carried out in our laboratory by Gavrilov. For the study an A vi-

rus (Shklyaver strain) was selected. In the first series of experiments the virus was neutralized by different dilutions of specific homologous immune serum, after which it was introduced into the allantoic cavity of chick embryos. For the next passage allantoic fluid of embryos was taken which corresponded to the first dilution of serum which did not provide complete virus neutralization, etc. After 20 such passages the virus did not alter its antigenic structure and remained identical to the original. No changes occurred even after 30, 40, and 50 passages. Therefore, the experiments did not confirm the data of the previous authors: the serum by itself, reacting with the virus in vitro (that is, in an inactive state), did not alter the antigenic structure of the influenza virus.

Different data were obtained from virus passages through the bodies of immunized mice. The mice were first immunized intraperitoneally by live virus and after two weeks were infected intranasally with different doses of virus. In connection with the quite strong immunity the passages were possible only with the injection of massive doses of the virus. For the purpose of obtaining passaged material the mice were sacrificed at various intervals -- 48, 72, 96 and 120 hours after the injection. In the latter case, serial passages could not be performed, because the virus was rapidly depleted.

In the first few passages the virus was not very pathogenic, and the mice carried an asymptomatic infection; with the increase in the number of passages the pulmonary involvement increased, and the mice died from influenzal pneumonia (Fig. 88).

Fig. 88. Extent of Pulmonary Involvement of Immunized Mice Depending on the Number of Passages Made by the Virus Being Tested.

The shaded areas schematically represent the size of the foci of hepatization of pulmonary tissue (Gavrilov, 1954).

The antigenic structure of the variants obtained was

studied in cross agglutination-inhibition reactions, complement-fixation reactions, cross neutralization on mice, and cross tests of immunity.

In Fig. 89 the results are presented of the study of the antigenic structure of the variants obtained in the hemagglutination-inhibition reaction, and in Fig. 90, in the cross neutralization experiments in mice.

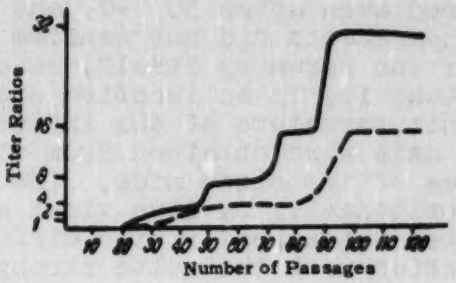

Fig. 89. Antigenic Relationships Between Original Virus and Its Variants Which Had Undergone Different Numbers of Passages Through the Bodies of Immunized Mice as Shown by the Hemagglutination Reaction.

_____ ratio of homologous serum titers against original virus to its heterologous titers (in the reaction with variants); ------- ratio of homologous serum titers against variants to their heterologous titers (in reaction with original virus) (Gavrilov, 1954).

The experiments of cross immunization in mice produced very similar results. Thus, mice immunized with the original virus were resistant to 31,000 doses of this virus and only to 10 doses of the strain variant which had undergone 90 passages. Correspondingly, the mice immunized with the strain variant had a resistance coefficient to it equal to 10,000, whereas that to the original strain was 56.

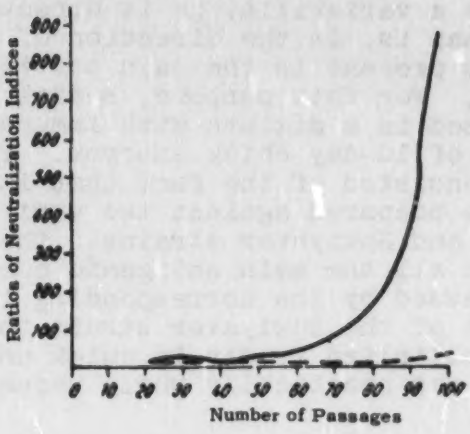

Fig. 90. Antigenic Relationships Between Original Virus and Its Variants According to the Data of Cross Neutralization Experiments on Mice.

——— ratio of homologous neutralization indices of sera against original virus to its heterologous indices; ------- ratio of homologous neutralization indices of sera against variants to their heterologous indices (Gavrilov, 1954).

In Fig. 91 the relationship is shown between the antigenic structure of the original strain and its variants and the prototype strains of influenza viruses.

On this Figure one fact attracts attention. As is well known, the Shklyaver strain has antigenic groups of the PR8 virus. These groups were not affected by the immunity and, therefore, remained unchanged. The changes occurred exclusively with the type WS antigen (the main antigen in the Shklyaver strain). These data indicate the adequacy of the change in antigenic structure with respect to the effect of the medium, that is, in the given case the effect of the specific immunity.

The variants obtained proved to be stable -- they completely preserved their new antigenic structure after repeated passages through the bodies of non-immune mice. Repeated control passages of the original virus through the bodies of immune mice which were conducted in parallel with this did not change the antigenic structure of the virus either.

Solov'yev and Gutman (1954) made an attempt at creating experimental conditions wherein the immunity factors

might act on the antigenic structure of the influenza A virus and produce a variability in it directed toward the A-prime virus, that is, in the direction of the serological type which at present is the main participant in the epidemic process. For this purpose, serial passages of the virus were used in a mixture with immune sera in the allantoic cavity of 10-day chick embryos. The method of virus passages consisted of the fact that immune sera (of guinea pigs) were prepared against two varieties of the A virus -- the PR8 and Shklyaver strains. Therefore, in these experiments all the main antigenic components of the virus were suppressed by the corresponding immune sera. After 10 passages of the Shklyaver strain the strain variants obtained multiplied freely in chick embryos in the presence of what was practically whole serum.

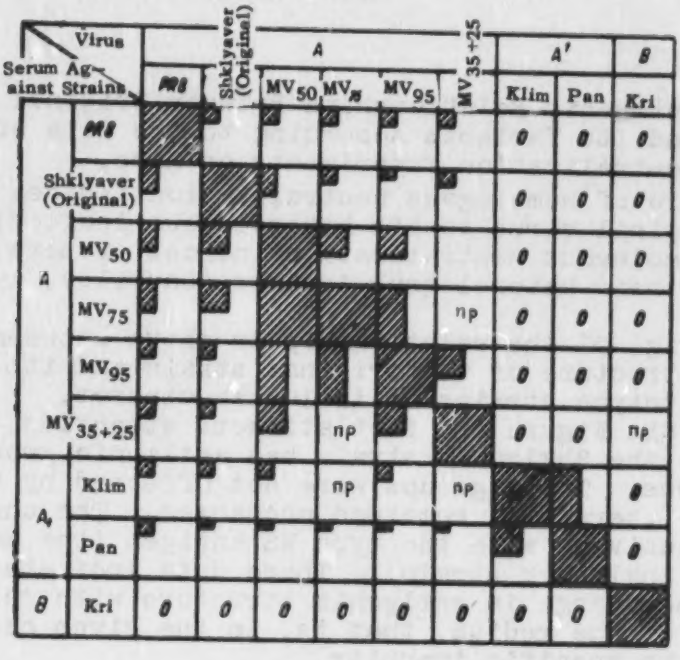

Fig. 91. Antigenic Relationships Between Original Shklyaver Strain, Its Variants and Representatives of the Main Serological Influenza Virus Groups.

The completely hatched square corresponds to the homologous titer taken as unity or to the ratio of the heterologous to the homologous titer when they coincide; the hatched area of the square corresponds to a fraction obtained by dividing the heterologous serum titer by the homologous titer. Zero -- ratio of 1/64 or lower; np -- reaction not performed (Gavrilov, 1954).

For the purpose of obtaining an appropriate strain variant of the virus 15 passages were required. However, whereas the Siklyaver strain returned to its original condition after five passages without serum, the newly acquired properties in the PR8 strain proved to be stable and were maintained after 25 passages without serum.

For the purpose of solving the problem as to the direction in which the change in antigenic properties of this strain variant (Pr8-u) proceeded cross-neutralization experiments with the standard A, A-prime and B sera were performed.

As a result, the artificially obtained variant showed its greatest similarity to the A-prime virus, specifically to the strain isolated in the spring of 1949 YeM prime strain. The reasoning for this conclusion was based on cross hemagglutination-inhibition experiments and cross neutralization of viruses in chick embryos (Table 48).

Table 48

Cross Neutralization of Pr8-u, PR8 and YeM-Prime Strains in Hemagglutination-Inhibition and Neutralization Reactions

Strains / Immune Sera Against	Hemagglutination–Inhibition Reaction			Neutralization Reaction in Embryos		
	PR8-u	PR8	YeM$_1$	PR8-u	PR8	YeM$_1$
YeM$_1$ (A$_1$)	1:160	0	1:640	1:320	0	1:640
PR8 (A)	1:10	1:640	0	0	1:640	0

The figures represent the highest serum dilution which can neutralize the hemagglutination and multiplication of the virus in embryos.

These serological data have been confirmed by experiments of cross immunity testing in which mice participated which had been vaccinated with the Pr8, YeM-prime strains and the artificially obtained PR8-u variant. The results of these experiments are presented in the form of a protocol (Table 49). The infection of the vaccinated animals was accomplished by mouse-adapted strains.

Cross immunity testing showed that mice vaccinated with the YeM-prime strain and the PR8-u variant are protected to the same degree against infection by the YeM-prime

virus, and that they do not have any immunity against infection with a PR8 virus.

Table 49

Cross Immunity Testing

Tests of Immunity With Strains	Virus Dilution for Infection	Mice Immunized by Strains			Control (Infection of Non-Immunized Mice)
		YeM	PR8	PR8-u	
A_1 (EM_2)	10^{-1}	0/4	4/4	0/4	4/4
	10^{-2}	1/4	3/4	0/4	4/4
	10^{-3}	0/4	3/4	0/4	3/4
	10^{-4}	0/4	1/4	1/4	1/4
	10^{-5}	0/4	0/4	0/4	0/4
	10^{-6}	0/4	0/4	0/4	0/4
A (PR8)	10^{-1}	4/4	0/4	4/4	4/4
	10^{-2}	4/4	0/4	4/4	4/4
	10^{-3}	3/4	0/4	3/4	3/4
	10^{-4}	3/4	0/4	3/4	3/4
	10^{-5}	1/4	0/4	0/4	0/4
	10^{-6}	0/4	0/4	0/4	0/4

The denominator represents the total number of mice; the numerator, the number of mice which died from influenza.

Therefore, the experiments described constitute evidence to the effect that conditions may be created in the laboratory wherein the immune medium determines the change in the hereditary properties of the virus. It is important to note thereby that the experimentally produced variability in its antigenic structure proceeds in the same direction as is observed under natural conditions.

The results of these investigations confirm the hypothesis that the variability factor in the antigenic structure of influenza viruses is a specific immunity, whereby the most distinct data are obtained when the influenza virus is acted on by the entire immune organism or by a sum of specific immunity factors (complete set of antibodies). These changes are of an adaptive nature and are entirely adequate to the effect of the medium, that is, of the immune organism.

33. Evaluation of Facts of the Variability of Influenza Virus Antigenic Structure and Criticism of Certain Concepts

Facts which have been accumulated at the present time pertaining to the variability of influenza virus antigenic structure are of importance not only for the solution of the influenza problem but also for many divisions of general virology, biology and epidemiology.

The phenomena of variability of the antigenic structure of influenza viruses could not help but attract the attention of many investigators, and in recent years attempts have been made repeatedly in the Soviet and foreign literature to interpret and generalize the facts observed.

Of the statements of foreign virologists the views of Burnet, Hirst, Andrewes, Horsfall and Smith, published in their articles which review and generalize on their investigations, deserve analysis.

Isaacs and Andrewes (1951) were the first in the foreign literature to evaluate and understand the ubiquitous changes in the antigenic structure of influenza viruses. After noting the progressive changes in the antigenic structure of these viruses circulating among the population and the disappearance of the old strains (WS, PR8 types), they directed attention to the P-Q variation of the influenza viruses described by Mulder, and attempted to associate them with the characteristics of influenza distribution. In their opinion, in 1950/51 two types of influenza distribution were observed: small epidemics which, however, consisted of numerous outbreaks which were particularly characteristic of this period in the Scandinavian countries, and considerable epidemics imported from without and spreading along communication routes, an example of which was the influenza epidemic in Liverpool. In comparing these data with the analysis of the antigenic structure of the influenza virus strains isolated the authors found that the viruses isolated during the epidemics of the Scandinavian type belonged to the Q group, while the viruses isolated during the epidemic of the Liverpool type belonged to the P group. Hence, they came to the conclusion that the influenza virus in the Q phase, while not very immunogenic, survives better in the bodies of immune persons, becoming an endemic virus; its being in the P phase is characteristic of the epidemic or pandemic distribution of influenza.

It is not hard to show the error of this hypothesis. The experimental data of Gorbunova, who showed how unfounded attempts were to tie in the P-Q phases of the influenza virus with the immune state of the organism wherein its multiplication is occurring and how arbitrary the very separa-

tion of the P-Q variants is as phases in the development of the virus, have been presented above. The careful study of Mulder's material and that of the authors of the hypothesis being analyzed does not permit us to connect the P-Q phases of the influenza virus with the type of spread of the epidemics either. As a matter of fact, in 1949 in Holland during an influenza epidemic all three types (P, Q, R) of influenza viruses were isolated, and during the influenza pandemic of 1950-1954 in South Africa and Australia, from where the epidemic of the Liverpool type had come in the opinion of Isaacs and Andrewes, both the P- and Q-types of virus were also isolated; they have also been isolated in Europe. Therefore, there is not a single fact which confirms the hypothesis being analyzed.

However, this hypothesis is untenable for two other reasons.

First, it diverges from attempts to explain the variability in the influenza virus antigenic structure really observed. Indeed, the hypothesis of the P-Q variations assumes a kind of closed circle of phasic transformations of the influenza virus: the P-phase produces the epidemic, and when general immunity occurs the virus changes into the Q-phase, in which it is found until the immunity of the population weakens. If such is the case why then does a change occur in the antigenic structure of the viruses into which the previously circulating strains were divided? Isaacs and Andrewes circumvent these questions.

Secondly, the hypothesis being analyzed attempts to explain the cause of influenza epidemics through only some of the changes in the biological properties of the virus, without even putting the question of the causes of these changes. This is a variant of an old theory which has been rejected long ago, which explained the causes of epidemics by the variations in the virulence of the microbe, a theory which attempted to reduce the complex epidemic process to a single microbial factor, a theory in which the cause (epidemic process) is taken for the effect (variability of the virus).

Everything which has been stated permits us to reject Isaacs and Andrewes conception decisively as being without scientific foundation.

Horsfall (1952) regards the progressive changes in the antigenic structure of influenza viruses as the result of the biological selection of antigenic variants in the immune organism. He circumvents putting the question of the causes of occurrence of the variants, although from the text of his article it may be understood that they occur spontaneously, regardless of the effect of the immune organism. One can only wonder about the degree to which Weis-

mann's theory is interfering with this author's gaining a proper conception of completely obvious facts of the variability of the antigenic structure of influenza viruses under the influence of specific immunity.

Smith (1952) advanced his own conception concerning the structure and functional plasticity of the influenza virus, of which we shall analyze here the part which pertains to the variability of the antigenic structure. The author notes that in the past 20 years a definite evolution of the influenza A-A-prime viruses has occurred, namely, in place of the stable antigenic types a sequential shift of the WS, PR8 and A-prime viruses has occurred during this period of time. Various correct statements are made in his work. Thus, the author speaks of the adaptive nature of the influenza virus variability during the process of passage of it in mice. The author's idea is correct that, after adapting to the bodies of various animals, the virus assimilates the host proteins which become a component part of it. The very term "plasticity," which the author introduces for the purpose of characterizing the phenomena of influenza virus variability, indicates that he considers them an adaptation to environmental conditions. Smith distinguishes four types of structural plasticity of influenza viruses: the acquisition and loss of antigens (A and A-prime viruses), change in the proportions of the antigens (variants of the A viruses), change in the surface configuration of the receptors (Francis phenomenon) and assimilation of the host proteins (mouse and egg variants of the virus). Unfortunately, Smith's conception suffers from essential defects as a whole. First, the author analyzes the changes in the antigenic structure and other biological properties of the influenza virus as spontaneous mutations, although he himself speaks of the adaptation to the new environment in which these mutations are occurring. Secondly, the acquisition and loss of antigens and the change in the proportions of them are represented by him mechanistically, as a movement around a tight circle, rather than as a development. It is sufficient to state that in his system the influenza A and A-prime viruses are represented by the symbol A $\rightleftarrows$ A-prime, as interchangeable mutants. In this connection, Salk develops very similar views (1952): in the phenomena of influenza virus variability he sees only a combination, in various proportions, of the limited number of antigenic components included in the virus particle. Thirdly, he does not even attempt to find the causes of the changes in the antigenic structure of influenza viruses in the epidemic process, nor does he even analyze the epidemic process itself as a factor in variability. In sum, threfore, the various correct state-

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ments made are devaluated by the general incorrect and unilateral treatment of the variation phenomena.

The examples presented are very instructive. They constitute evidence that the facts which apply to the variability in the antigenic structure of influenza viruses are automatically stimulating many investigators to evaluate them as phenomena of an adaptive nature. At the same time, the unilateral treatment of the variation phenomena and the absence of a correct epidemiological theory do not permit these investigators to evaluate correctly the facts which they are observing. Unaware of it themselves, these investigators are permitting gross contradictions in the views which they are expressing.

From this point of view, it is expedient to analyze the attempts of Burnet and Hirst to explain the facts which they observed in the passages of mixed influenza virus cultures.

In summarizing the results of the investigations of the reaction of viruses in mixed cultures, Burnet (1953) treats them from the point of view of the gene theory. The forms of viruses obtained, which have the properties of both strains (parent strains) is considered to be a "gene recombination," and he presents formulas for the exchanges of genes. Thus, in his opinion, if we designate the MEL strain by the formula ABCDE and WSN strain by the formula abcdF (the experiments were performed in chick embryos), then the "mutant strains" occurring as the result of gene recombination will have the formula: M + = ABcDeF and WS — ef = abCdEf. Hence, he draws the conclusion that the Cc and Ee factors are transmissible, and in the given case a genetic reaction occurs. In another work (Lind and Burnet, 1953) the recombinants M+ and WS+, distinguished serologically and by other properties, were obtained as the result of the reaction of the MEL and WSE viruses, and then, as a result of the reaction of these recombinants, inverse recombinants were obtained which in their properties were similar to the original MEL and WSE strains.

Careful examination of the factual material presented in the works mentioned permits us to draw the conclusion first that there is a lack of correlation between Burnet's explanation mentioned and the factual material which he obtained. As a matter of fact, the recombinants obtained, which have a number of properties of the parent strains, at the same time possessed a number of other properties which were absent from the parent strains. These properties (for example, the unusual degree of thermoresistance of the hemagglutinin, which was higher than in the parent strain with a thermoresistant hemagglutinin) could not have gone unnoticed by the experimenters them-

selves. Therefore, the consideration of the strain variants as the result of the mechanical combination of various characteristics of the parent strains simply does not correspond to the facts. Even with these reservations the facts observed by no means fit into the framework of the gene-mutation theory and, moreover, contradict it, because the viruses have no sexual process. Therefore, Hirst, working with the A and B viruses (Gotlieb and Hirst, 1954) and who also take the positions of formal genetics, come to the conclusion that in the given case there is a "phenotypic mix-up". In his subsequent works Burnet (1954) had to admit that in the experiments with virus recombination too many deviations are obtained to explain them from the standpoint of the gene theory (Burnet and Lind, 1954). After this, what remains of "gene recombination" and of attempts to explain the phenomena observed by the gene-mutation theory? Even in this case the erroneous theory enters into an unsolvable contradiction with the factual material.

If we direct our attention to the last summary by the foreign investigators concerning the variability of influenza viruses, in which the generalizing articles of Burnet (1954) and Magnus (1954) are represented, we find nothing new here either. The entire discussion centers around the theory of permanent mutations, combinations of a limited number of antigenic components, selection of antigenic mutants by incomplete immunity and gene combination factors, that is, everything is in this cycle of concepts of limited variability in the form of combination of permanent characteristics.

The facts observed of the variability of influenza virus antigenic structure can obtain their correct explanation only from the standpoint of creative Darwinism. Such attempts have been made in recent years by Zhdanov and Solov'yev (1951, 1952), Zhdanov (1953), Gorbunova (1953), and somewhat earlier were expressed in a general formula by Smorodintsev (1948).

The latter believes that the influenza virus is plastic and accessible to a deep-seated effect of definite environmental factors and that the immunological effect on virulence and the antigenic structure of the virus are of principal importance. These conclusions were made chiefly on the basis of work of Andrewes (1942) and were not confirmed by his own factual material. In the works of Zhdanov and Solov'yev the factual material which has been accumulated in recent years in the USSR and abroad have been analyzed. Below, an attempt is being made at a more systematic presentation of the views which they developed. In explaining the phenomena of variability of the virus antigenic structure a start should be made from the standpoint of

Michurinist biology concerning the unity of the organism in the environment, the production of phenomena of variability by environmental influences, the adequacy of these changes to the environmental changes, and the adaptive nature of variability.

From this point of view, on the one hand, the reresistance of the antigenic structure of influenza viruses becomes clear when they are cultivated in the bodies of various animals, as does also the effect on them of various physical and chemical factors, and, on the other hand, clarity is introduced into the lability of the antigenic structure of influenza viruses when they are passaged in an immune organism.

Passages through the bodies of various animals have an influence, as will be seen below, on many biological properties of the viruses. To a lesser degree this applies to the effect of various physical and chemical factors, because it is not expressed directly in connection with the virus but rather chiefly through the organism (or tissue) of the host as the result of the obligate parasitism of the influenza virus.

The environmental changes mentioned usually do not affect the antigenic structure of influenza viruses, and specifically have no effect on the antigenic composition of its hemagglutinin, apparently because the substrate on which the virus acts with its own antigens remains essentially unchanged in these cases (let us recall that the influenza virus reacts with the erythrocytes of many biological species which are very distant from one another phylogenetically). It is another matter when the virus enters into the organism which is specifically immune to it. Here, it must either die or adapt itself to new altered conditions. Since this adaptation can occur only by means of overcoming the specific immunity, the changes in the biological properties of the virus during the course of adaptation to life in the immune organism primarily and chiefly involve its antigenic structure, because with the alteration of the antigenic structure the specific immunity stops acting on the virus and stops preventing its multiplication in full measure or partially, depending on the degree of changes in the antigenic structure which have occurred. Therefore, in the given case changes in the biological properties of the virus, as is the case with all other expressions of its variability, are strictly adequate to the changes in the environment. Under the influence of a specific immunity the antigenic structure of the virus is changed.

The fact that the antigenic structure of the hemagglutinin is changed is also entirely to be expected, be-

cause it represents the apparatus by means of which the virus comes into contact and reacts with the immune cell or tissue.

We believe that this manner of considering the experimental data (resistance of the antigenic structure of influenza viruses in passages through a non-immune organism of various biological species and its variation in passages through the specifically immune organism) is also applicable for the explanation of the variation in the antigenic structure of viruses observed in the epidemic process.

The extensive distribution of influenza among the population leads to the fact that the majority of people are in a condition whereby they have a more or less definite immunity to influenza. In connection with the incompleteness of the immunity to influenza, the virus can multiply in the bodies of people who havepartially lost their immunity.

Actually, this leads to the fact that during the course of the epidemic process the virus more frequently enters an immune than a non-immune body, and, therefore, it constantly is being subjected to the effect of specific immunity. In adapting itself to parasitization in the immune organism and overcoming the specific immunity in the course of this adaptation, the influenza virus changes its antigenic structure, which in its turn has an influence on the specificity of the immunity of the population. Therefore, in the course of the epidemic process a continuous change occurs in the antigenic structure of influenza viruses circulating among the population. By accumulating gradually these fine quantitative changes periodically leads to large-scale qualitative changes -- to the appearance of new antigenic varieties (serological types) and influenza virus species. An example of this species-forming process is the appearance in 1945-1947 of the new influenza virus species -- the A-prime virus. Therefore, as Gorbunova indicates, the influenza virus in man is an example of such a biocoenosis in which the parasite determines the immunobiological changes in the host organism, which in their turn become the formative mechanism for the occurrence of new serological types of the virus.

It seems to us that the study of the variability of the antigenic structure of influenza viruses makes it possible for us to understand certain facts which apply to other virus infections.

Certain applications of this hypothesis to problems of immunology and epidemiology of influenza will be analyzed in the appropriate chapters. Here, it remains for us to give a brief evaluation of the data obtained from pas-

sage of influenza viruses in mixed cultures.

The untenability of Burnet's and Hirst's attempts to explain the facts which they observed has been shown above. We believe that the only correct way of considering these phenomena is that they represent vegetative hybridization. This concept was incorrectly applied by Kalina (1952) for the purpose of explaining changes in the antigenic structure of bacteria as a result of their assimilation of the constituents of bacteria of other serological types. In this case, this explanation should be considered correct, because we are dealing with the combined cultivation of two species or varieties of viruses and the interaction of them. The variety of strain variants obtained thereby, which do not fit within the framework of genic lines, reflects the complexity of interaction of viruses with one another in the body or tissues in which their cultivation is occurring, and the presence of both unstable variants and variants in which the properties are firmly reinforced and transmitted to subsequent generations is very characteristic of the phenomena of vegetative hybridization and reflects a greater or lesser adaptation of them to given conditions of the medium in which they are. The phenomena of vegetative hybridization of influenza viruses are not only of theoretical interest but they can also find application in the preparation of vaccine strains.

34. Immunogenic Properties

The rules and regulations of the change in immunogenic properties of influenza viruses have been studied in detail and understood by Soviet virologists, and their priority in this matter is completely indubitable.

In the foreign literature on this subject there are only snatches of scattered data without any serious attempts to generalize them. Thus, Briody (1950), Mulder and Brams (1952) indicate the greater antigenic properties for mice of strains which have been passaged in mice than of those strains passaged in chick embryos; Isaacs and Sampaio (1953) have noted the greater immunogenic properties of formol vaccine prepared from mouse strains for mice than formol vaccine prepared from egg strains.

On the other hand, the investigations of Burnet (1937) are well known; he established the fact that the influenza virus strains adapted to chick embryos, while not pathogenic for mice, at the same time immunized mice well (after intranasal inoculation) against large doses of the active mouse virus. These egg strains arehighly immunogenic for ferrets. These and several other such works exhaust the foreign literature on this problem.

In the Soviet literature this problem has been studied in detail in connection with the putting of the problem of obtaining effective live vaccines. In 1949, Zil'-ber (1949), evaluating his own works and those of his colleagues, noted that the immunogenic properties of laboratory strains of influenza viruses cultivated in mice and chick embryos are very small, and he connected the poor immunogenic properties of laboratory strains with the loss of the ability to survive and multiply actively in the human nasopharynz. He pointed out a method of cultivating influenza viruses in human tissue cultures as the most promising method for obtaining highly immunogenic strains.

The systematic development of this problem has been accomplished by Zhdanov, Solov'yev, Smorodintsev and their co-workers.

Investigations which have been made in the Institute of Virology of the Academy of Medical Sciences USSR have been generalized in the works of Zhdanov (1953, 1954), Zhdanov and Solov'yev (1954), Fadeyeva (1952, 1953), Zakstel'skaya and Ritova (1953, 1954) and Slepushkina (1954).

Fadeyeva made a comparative study of the immunogenic properties of influenza virus strains cultivated in the bodies of chick embryos as well as in the cultures of mouse and human embryonic lung tissue. A description of the changes in the biological properties of the viruses during the course of adaptation to these media will be presented in the following sections; here, we are presenting data concerning their immunogenic properties.

The testing of the immunogenic activity of the strain variants was accomplished by the infection of immunized mice with different doses of a strain variant of the same antigenic type highly virulent for mice.

In the first series of experiments a study was made of the immunogenic properties of the strain variants of the A virus (Shklyaver). The results of these experiments are shown in Fig. 92.

Mice immunized by the Shklyaver $Ya_{24}TECh_{30}Ya_1$ strain variant withstanding 1175 LD_{50} of a pure mouse strain, which speaks for a considerable change in the immunogenic properties when cultivating the strain in heterogenous tissues outside the body.

In the second series of experiments a study was made of the immunogenic properties of the strain variants of A-prime virus (S6). The data of this series of experiments are presented in Fig. 93.

As is seen from Fig. 93, the mice which were prepared by immunization with the $S\text{-}6Ya_7M_{18}Ya_2$ strain resist more than 10,000 LD_{50} of the homologous strain. Mice immunized with pure egg line $S\text{-}6Ya_{10}$ resist only 23 LD_{50} of the

S-6$Ya_7M_{18}Ya_2$ strain, whereas immunization of mice with the tissue strain variant S-6$Ya_7TME_{18}Ya_2$ gives a good effect, protecting the mice against 1047 LD_{50} of the strain S-6$Ya_7M_{18}Ya_2$.

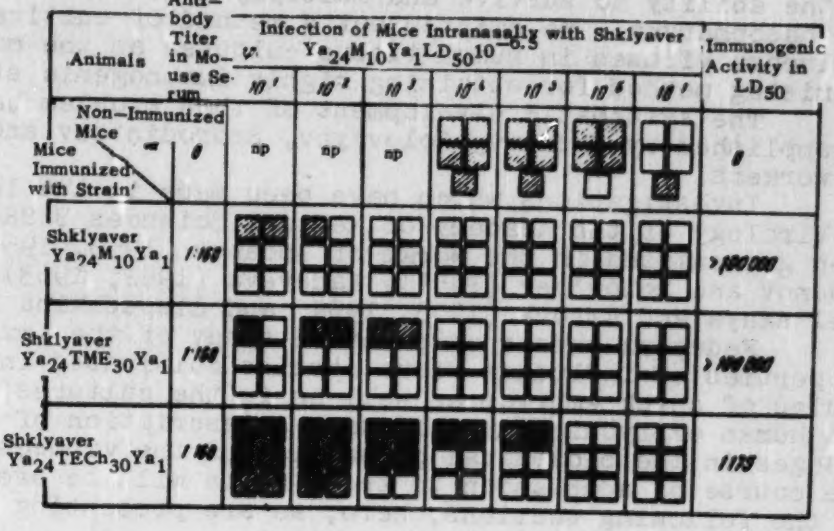

Fig. 92. Experiments with Immunization of Mice with Strain Variants of A Virus (Shklyaver) Passaged Through Mice (M_{10} Passages, in Tissues of Mouse Embryos (TME_{30} Passages) and Tissues of Human Embryos ($TECh_{30}$ Passages).

The hatched boxes represent mice which died; the white boxes represent surviving mice; np -- experiment not performed; Ya -- egg passages; M -- passages in mice (Fadeyeva, 1952).

It should be noted that the tissue and pure mouse variants of S-6 underwent two allantoic passages in developing chick embryos before testing.

The results of the two series of experiments on the study of the immunogenic properties of the strain variants speak for essential changes in these properties during the course of adaptation of the virus both to the intact organism and to tissue cultures, whereby these changes depend on the species of animal or the nature of the tissue cultures. The adaptation of influenza virus to lung tissue of

mouse embryos is accompanied by an increase in the immunogenic activity against the variant which caused the death of the mice.

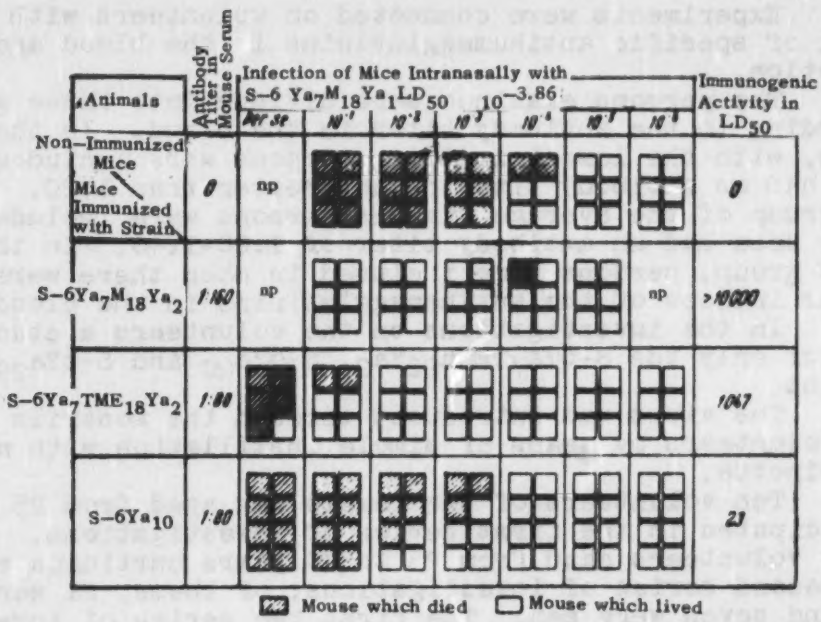

Fig. 93. Experiments with Mouse Immunization with Virus A-Prime (S-6) Strain Variants Passaged in Mice (M_{18} Passages), in Mouse Embryo Tissues (TME_{18} Passages) and in Chick Embryos (Ya_{10} Passages). The key is the same as for Fig. 92 (Fadeyeva, 1952).

By means of the reaction of cross testing of immunity on white mice it was possible to detect essential differences in the strain variants which were expressed in different immunogenic activities with respect to the strain of the same antigenic type pathogenic for mice.

Subsequent investigations were directed at a study of the immunogenic properties of different strain variants for man.

The activity of survival and duration of multiplication of the virus on the mucous membranes of the respiratory passages and the capacity of the strain for stimulating the production of specific antibodies in the blood and in the human nasal secretion were investigated. Great importance in the work was ascribed to the dosage of the material investigated.

A study was made of the same antigenic group, which had undergone a large number of passages in developing chick embryos, in comparison with the ordinary laboratory strain.

Experiments were conducted on volunteers with a low index of specific antihemagglutinins in the blood and nasal secretion.

The persons examined were divided into three groups according to the antibody titer in the blood. In the first group, with the lowest indices, persons were included whose sera had an antibody titer of no greater than 1:20. In the group of the average figures persons were included whose sera had an antibody titer of 1:20-1:40. In the third group, persons were included in whom there were higher indices of the antihemagglutinins in the blood.

In the investigations on the volunteers a study was made of only the S-$6Ya_7TECh_{22}Ya_2$, S-$6Ya_{20}$ and S-$6Ya_{29}$ strains.

The virus was introduced through the mostrils of the volunteers by means of simple instillation with a sterile pipette.

Ten volunteers of the female sex aged from 25 to 40 participated in the first series of investigations. Twenty-eight volunteers aged from 25 to 40 years participated in the second series of investigations; of these, 21 were women and seven were men. The first two series of investigations were carried out during the interepidemic period from July through December 1951. In the third series of investigations which were carried out in the hospital of the influenza clinic of the institute of virology of the Academy of Medical Sciences USSR, 30 volunteers aged from 20 to 30 years participated; of these 14 were women and 16 were men. The biological properties of the strain variant S-$6Ya_7TECh_{22}Ya_2$ in the third series of investigations were checked in conjunction with our scientific colleagues in the Institute, Ritova, Dreyzin and Slepushkin. Clinical observations on volunteers were made by scientific workers in the clinic headed by Epshteyn. This series of investigations was carried out in the pre-epidemic period in January 1952.

The capacity of the virus for multiplying on the mucous membrane of the upper respiratory tract of man was determined by means of the investigation of washings for the presence of variable virus. The results of these experiments are presented in Figs. 94 and 95.

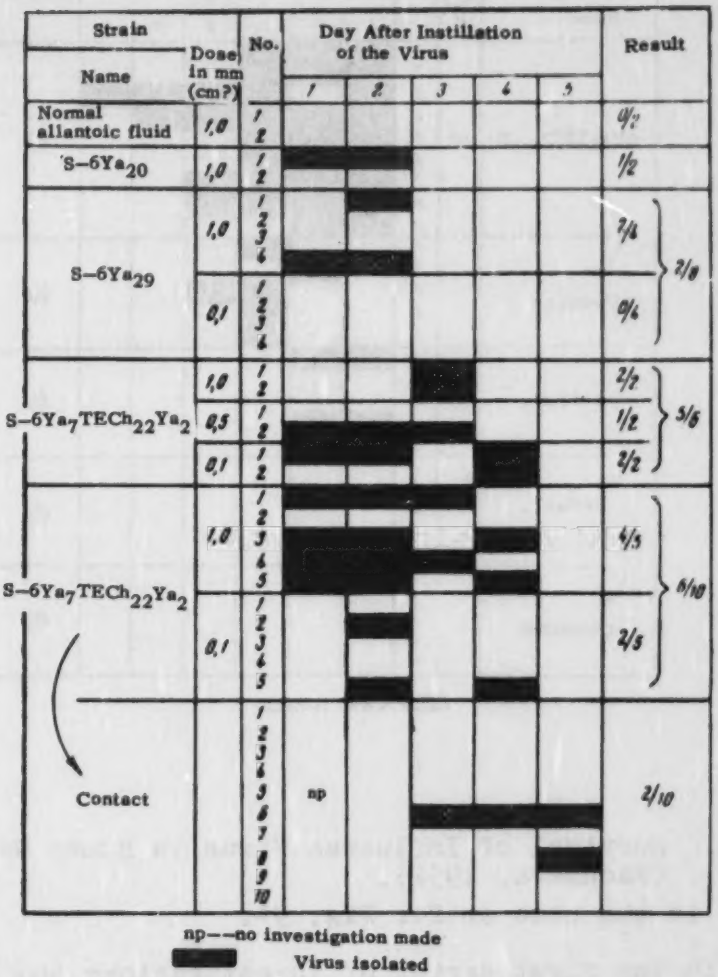

Fig. 94. Survival of Influenza Virus in Human Nasopharynx (Fadeyeva, 1952).

The numerator represents the number of persons in whom the virus was isolated; the denominator, the total number of those examined.

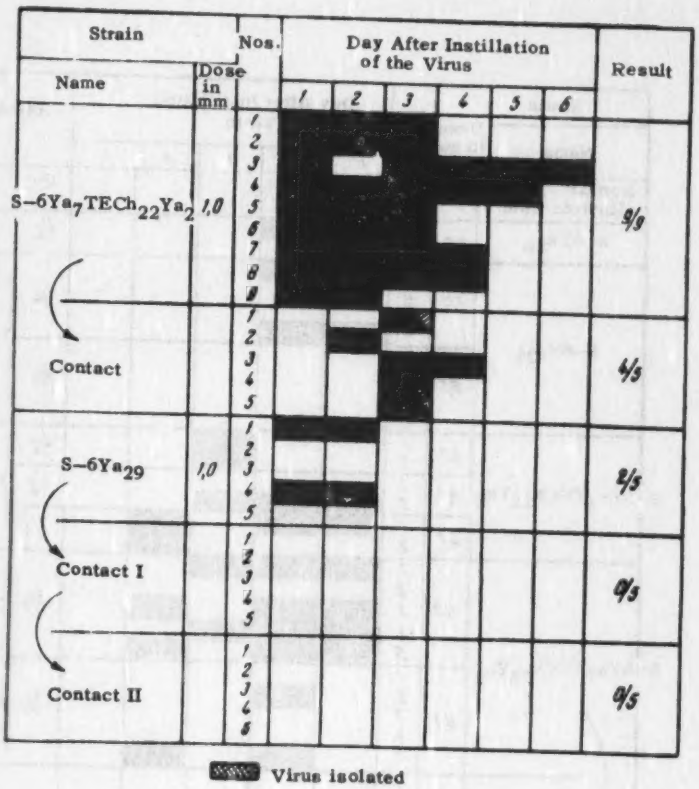

Fig. 95. Survival of Influenza Virus in Human Nasopharynx (Fadeyeva, 1952).
The key is the same as for Fig. 94.

In the first series of investigations the S-6Ya$_{20}$ strain was inoculated into two volunteers (LD_{50} = 10^{-7}; the hemagglutination-reaction of allantoic fluid with the chick erythrocytes was positive in a dilution of 1:160). Five-tenths of a cubic centimeter of a whole allantoic virus culture was instilled into each nostril. The S-6Ya$_7$TECh$_{22}$Ya$_2$ strain was inoculated into six other volunteers (LD_{50} = 10^{-7}; the hemagglutination reaction of the allantoic fluid was positive in a dilution of 1:160), whereby two of the volunteers were inoculated with a whole allantoic culture; two, in a dilution of 1:2; and two, in a dilution of 1:10. The virus dilutions were prepared in phys-

iological solution. It was possible to isolate the virus after two days from one of the volunteers who received the S-6Ya$_{20}$ strain. Of the six volunteers who had received the strain variants S-6Ya$_7$TECh$_{22}$Ya$_2$ the virus was found in five persons. The isolation of the virus was noted during the four days which followed the inoculation.

In the second series of investigations eight volunteers were injected with the S-6Ya$_{29}$($LD_{50} = 10^{-7}$; hemagglutination reaction of the allantoic fluid was positive in a dilution of 1:160) strain; four of them were inoculated with one cubic centimeter each of the whole allantoic virus culture; the other four, in a dilution of 1:10.

The next day after the inoculation the virus could be isolated in only one person. After two days the virus was isolated in two others; whereas of the 10 volunteers in whom the strain variant S-6Ya$_7$TECh$_{22}$Ya$_2$ had been inoculated ($LD_{50} = 10^{-7}$; hemagglutination reaction positive in a dilution of 1:160) the virus was found in six, whereby it was actively excreted for four days after the inoculation.

In the third series of investigations the S-6Ya$_{29}$ strain ($LD_{50} = 10^{-7}$; hemagglutination reaction positive in a dilution of 1:160) five volunteers were inoculated. The virus could be isolated in two persons during the first two days after the inoculation.

The strain variant S-6Ya$_7$TECh$_{22}$Ya$_2$ (LD $= 10^{-7}$; hemagglutination reaction positive in a dilution of 1:160) was inoculated into nine volunteers. In all nine persons viable virus was isolated from the nasopharyngeal washings, whereby the active excretion of this strain, as in the first two series of experiments, was observed for four days after the inoculation.

On the fifth day, the virus was recovered from two persons, and on the sixth day, from one.

Generalizing on all these observations it may be considered that in the control group of persons vaccinated with a pure egg S-6 strain virus from the nasopharyngeal washings was isolated in 30 percent in just the first 48 hours after the inoculation, whereas in the group of persons vaccinated with the tissue strain variant S-6 virus could be found in 80 percent of the volunteers, whereby in 40 percent the virus was isolated even on the fourth day after the inoculation. These observations emphasize the activity and the definite affinity of the tissue strain variant for the mucous membranes of the respiratory passages of man.

The dose of virus administed influences the nature

of multiplication of the virus on the mucous membrane of the human upper respiratory passages. After the inoculation of 0.1 cubic centimeter of the S-6Ya$_{29}$ strain it was impossible to find any viable virus in the nasopharyngeal washings of volunteers. The S-6Ya$_7$TECh$_{22}$Ya$_2$ strain administered in the same dose possesses the capacity of surviving on the mucous membrane, although it is isolated less regularly and not in all volunteers.

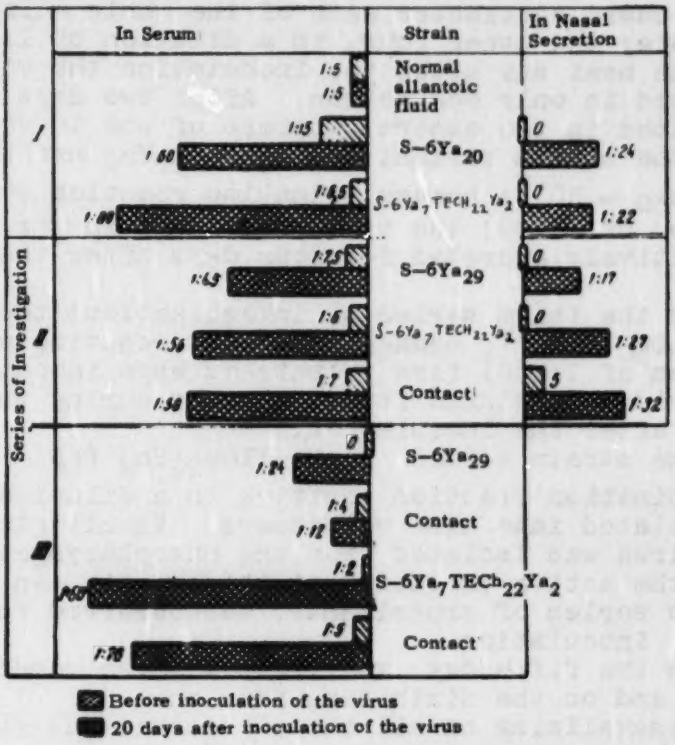

Fig. 96. Immunological Reaction in Those Vaccinated According to the Hemagglutinin Titer After the Nasopharyngeal Administration of Egg and Tissue Strains of Influenza Viruses (Fadeyeva, 1952).

In all the series of experiments an investigation of the blood was made, and in the first two series, of the nasal secretion also for the antihemagglutinin content both

before administration of the virus and 15-20 days after it. The results of these investigations are presented in Fig. 96.

From Fig. 96 it is seen that the single intranasal administration of both the ordinary egg strain S-6 and the tissue strain variant S-6 produce a considerable increase in the antibodies both in the blood and in the nasal secretion of the volunteers.

The tissue strain variant, however, produces a greater rise in the antibody titer than the simple egg strain. On analysis of protocols a rise in the antibody titer may be noted not only with respect to the homologous antigenic type A-prime but also to the antigenic type A.

It is not without interest to note that in contrast to the egg strain variants of the virus strain passaged in human tissues are transmitted by the contact route, being found in the nasopharynx of persons who have had close contact with those vaccinated. These characteristics of the tissue strains will be analyzed in greater detail in the chapter on prophylaxis. Here we should like to note that the tissue strains are not very pathogenic for man and do not produce any apparent general or local reaction in the volunteers.

Further investigations were made by Zakstel'skaya and Ritova (1953), Ritova and Zakstel'skaya (1954). These investigations, the main aim of which was obtaining standard vaccine strains, completely confirmed Fadeyeva's data. In Fig. 97 material is presented graphically which characterizes the immunogenic properties of the egg and tissue strains. It should be noted that the observations of Zakstel'skaya and Ritova were made on hundreds of volunteers and gave absolutely uniform results.

In the course of these investigations a curious fact was found. On prolonged maintenance of the tissue-adapted strains a weakening of their infectious (chick embryo titers) and immunogenic (survival in human nasopharynx) properties gradually occurred. The latter properties were more labile and decreased earlier than the virus titer fell in chick embryos.

From the data which have been presented it is clearly seen that the immunogenic properties of the strains are directly related to their capacity of survival and active multiplication in the human nasopharynx.

Very similar data have been obtained by Slepushkin (1953, 1954), who made a comparative study of the egg and tissue strains of the A, A-prime and B viruses. After confirming previous data, he showed, first that the greater the number of passages that a strain has undergone in a heterogenous medium the more difficulty it has in adapting

to human tissue and, secondly, that the adaptation process and the process of change in the immunogenic properties occur more rapidly in the A-A-prime viruses than in the B viruses.

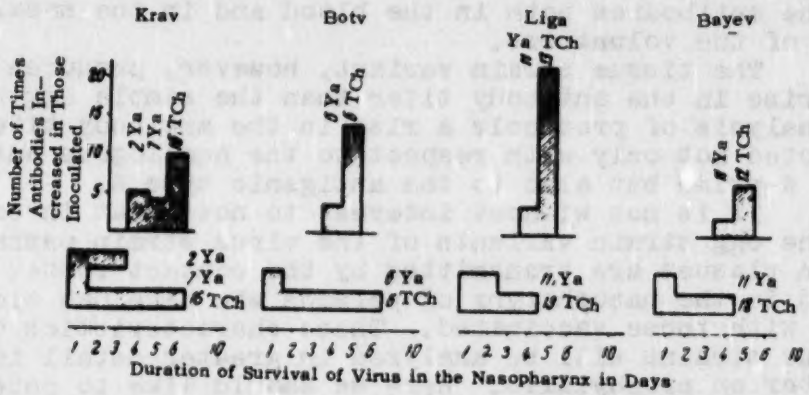

Fig. 97. Immunogenic Properties and Survival of Egg and Tissue Strains in the Nasopharynx of Various People.

The figures pertaining to the columns are the number of passages: Ya -- on chick embryos; TCh -- in human tissue cultures (Zakstel'skaya and Ritova, 1953).

The series of investigations made by Solov'yev and others (Solov'yev and Mariennikova, 1953; Solov'yev and Gutman, 1953) made it possible for the authors to come to very analogous conclusions. The authors showed that the freshly isolated strains which had undergone many passages in chick embryos survive well and multiply well in the human nasopharynx, give a distinct leukopenic reaction and produce an accumulation of specific antibodies in the blood and nasal secretion in high titers. The strains which have undergone a considerable number of passages in mice and chick embryos survive poorly, multiply in the human masopharynx and lose their immunogenic properties (Fig. 98).

Finally, mention should be made of the investigations of Smorodintsev and others (1948) who showed that the high degree of survival and immunogenic properties of the influenza viruses for man can be regained by the laboratory strains by means of passages of them through the human body.

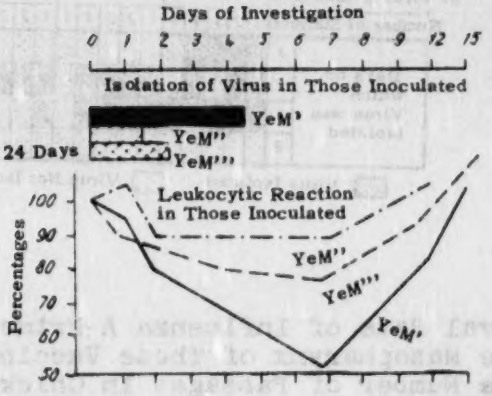

Fig. 98. Comparative Data on the Survival and Reactivity of the Strain Variants of the A-prime Virus Passaged in Mice (YeM'') and Chick Embryos (YeM''') Compared With the Original Strain, YeM' (Solov'yev and Gutman, 1953).

Sokolov's investigations (1954) contradict these data; while he accepts the fact that the influenza virus during the course of adaptation to the mouse organism loses its immunogenic properties for man he believes, at the same time, that adaptation to the chick embryo organism does not reduce its immunogenic properties. However, careful analysis of the experimental data presented by the author does not confirm this last conclusion. Thus, a virus A-prime strain (Kond) which has undergone 30 passages in chick embryos, does not survive so well in the human nasopharynx as the same strain which has undergone eight passages (Fig. 99). A strain of the A-prime virus (Liga) adapted to chick embryos did not survive so well as the strain adapted to human tissues (Fig. 100), although, as has afterwards been shown by Ritova and Zakstel'skaya (1954), this latter strain lost its immunogenic properties for man to a considerable degree after prolonged keeping. Correspondingly, the antibody titers in people immunized with tissue strains increased better than in those immunized with the egg strain.

Therefore, Sokolov's conclusion that prolonged cultivation of the influenza virus on chick embryos increases its antigenic and probably immunogenic properties cannot help but cause surprise, because it contradicts the author's own experimental data, not to mention the data of other investigators.

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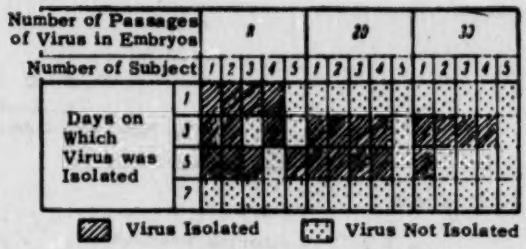
Number of Passages of Virus in Embryos
Number of Subject
Days on Which Virus was Isolated
Virus Isolated
Virus Not Isolated

Fig. 99. Survival Rate of Influenza A-Prime Virus (Kond) in the Nasopharynx of Those Vaccinated Depending on the Number of Passages in Chick Embryos (Sokolov, 1954).

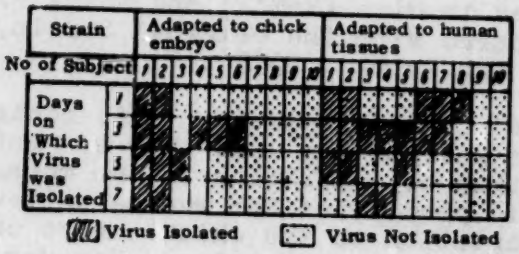

Fig. 100. Survival Rate of Influenza A-Prime Virus (Liga) Adapted to the Chick Embryo and Human Tissues (Sokolov, 1954)

For the purpose of understanding the process of change in the immunogenic properties of the viruses it is necessary to take into consideration the fact that they are closely associated with the process of adaptation of the strain to the new environment. A comparison of the data characterizing the adaptation of the influenza virus strains to tissue cultures of mice and man with the results of a study of the immunogenic properties of the viruses shows that the latter are stabilized only when the adaptation process of the virus to the tissue culture has been completed, which requires a considerable number of passages if the virus has previously been passaged in another medium

for a long time.

In summarizing the results of the study of the variability of the immunogenic properties of the influenza viruses certain conclusions may be drawn.

1. It should be considered an established fact that the immunogenic properties of the influenza viruses have species specificity. We cannot speak of a high degree of immunogenicity of various influenza virus strains generally -- such immunogenicity does not exist. The immunogenic strains can be the same for either man or for mice or for the ferret, etc.

2. Immunogenic properties of virus strains depend on the degree of their adaptation to a definite biological species. Thus, strains which are well adapted to mice are highly immunogenic for mice; strains adapted to man are highly immunogenic for man.

3. Thereby, the adaptation and, therefore, the increase in the immunogenic properties with respect to a definite biological species involve a decrease or even a loss of immunogenic properties for other biological species. On the strength of this fact egg strains of influenza viruses are characterized by a low degree of immunogenicity for mice, while egg and mouse strains are not at all or only slightly immunogenic for people.

4. The immunogenic activity is closely connected with the capacity of the strain for surviving and multiplying actively in the host organism. This is seen particularly distinctly in the study of the immunogenic properties of the influenza virus strains for man. Strains which have lost the capacity of surviving and multiplying in the human nasopharynx simultaneously lose their immunogenicity.

5. For the purpose of understanding the mechanism of acquisition and loss of immunogenic properties it is important to point out that the latter are determined by the species specificity of the host tissues. The influenza virus is immunogenic with adaptation not only to the whole organism but also to isolated tissue cultures. Thereby, completion of the process of adaptation of the influenza virus to a tissue culture of a given biological species coincides with the acquisition of the immunogenic properties for this species by the virus.

6. Apparently, the process of adaptation to a new medium represents a reorganization in the metabolic type of the virus, the adaptation of it to the tissue metabolism of a given biological species and an assimilation of proteins of the given biological species and, therefore, a reorganization of the heredity of the given species. This reorganization occurs by means of the uprooting of the old hereditary properties and the acquisition of new ones

which correspond to the new medium.

7. By virtue of the conservatism of heredity, when a virus is put into a new medium it maintains its old properties for several passages. This explains the fact that the influenza virus maintains its immunogenic properties for man and is capable of surviving in man during its first few passages in chick embryos. For this reason, viruses which have undergone many passages in chick embryos and mice adapt with greater difficulty to human tissue cultures and freshly isolated strains.

Changes in the immunogenic properties are not indisruptibly associated with pathogenicity. This problem will be discussed in greater detail in the next section; here we should like to note that in the process of adaptation to tissue cultures viruses which have previously lost their pathogenicity for man or for experimental animals do not regain it. Moreover, highly pathogenic strains gradually lose their pathogenic properties during the course of passages in tissue cultures even for the species in the tissue cultures of which the passages are being made.

The immunogenic properties of the influenza viruses are associated with their antigenic structure, but this association is unilateral. Strains which have a different antigenic structure are not immunogenic with respect to one another. This fact has been established from the time that the differences in the antigenic structure of influenza viruses became known. However, with an identical antigenic structure strains adapted to various biological species can possess a weakly expressed immunogenicity with respect to another host. Adaptation to various hosts, while not altering the antigenic structure, changes the immunogenic properties of the viruses.

The question arises -- what is the material substrate of the immunogenic properties of the viruses? Is it not some kind of additional antigen which is not detected by ordinary methods of serological investigations? Such an assumption is far from being without foundation if we take into consideration that adaptation of the virus to tissue metabolism of a given biologic species should be accompanied by the assimilation of species specific proteins. From this point of view the investigations of Smith, Belyavin and Sheffield (1953) deserve attention; they found a thermostable antigen inside virus particles which is common to A and B viruses and normal tissues of chick embryos and which is detectable by means of a complement-fixation reaction. However, for the purpose of solving this problem special, much more extensive and carefully performed investigations are needed.

35. Pathogenic Properties

Strains of viruses isolated from influenza patients are characterized to various degrees by pronounced pathogenic properties for laboratory animals.

In general, it may be stated that viruses of the A type are most pathogenic for white mice; viruses of the B type are least pathogenic, and viruses of the A-prime type occupy an intermediate position. Within the limits of each serological type considerable variations in pathogenicity for mice and other laboratory animals may be observed. As far as the type C viruses are concerned the strains studied proved to be non-pathogenic not only for mice but also for ferrets.

It is necessary, however, to point out immediately that the pathogenicity of influenza viruses for white mice and other laboratory animals is a property which may be acquired during the course of passaging the viruses in laboratory animals. This fact, which, by the way, is characteristic of other viruses, was noted immediately after the discovery of the influenza viruses, when white mice began to be utilized as a model for the study of this infection; they are less susceptible to influenza than ferrets. In the works of Smith, Andrewes and Laidlaw (1933) on ferrets, Zil'ber, Fal'kovich and Arkhina (1937) on white mice, Burnet (1936) in chick embryos it was shown that for the purpose of obtaining stable strains several passages are required in the course of which the influenza virus is adapted to a medium which is new for it, acquiring pathogenic properties for the new host. The same rules and regulations were established also in subsequent works with ferrets, mice, chick embryos, as well as in numerous works in which the pathogenic properties of the influenza viruses were established for various species of animals (hamsters, hedgehogs, field voles and others). Therefore, there is no sense in returning to the investigations mentioned and it is much more expedient to analyze certain rules and regulations in the increase of pathogenic properties of the influenza viruses during the course of their adaptation to the bodies of laboratory animals.

As has already been mentioned, within the limits of the same serological types various strains are characterized by different capacities for adapting themselves to animal organisms and different degrees of pathogenicity which can be acquired in this way.

The A viruses adapt most easily to the bodies of mice. Usually, after several (two or three) intranasal passages the A viruses begin to produce characteristic pathology in the lungs -- first in the form on scattered foci

and then in the form of a lobar hemorrhagic pneumonia. At the same time, various strains produce a fatal pneumonia beginning with the first few passages, and isolated ones, after a prolonged passage. Thus, according to the data of Stuart-Harris and coauthors (1940), strains isolated in 1939 in ferrets adapted to mice (became pathogenic for them) after different numbers of passages: the majority after two, frequently after four, and one, after fourteen passages.

The A-prime viruses are less pathogenic for mice than the A viruses. However, Ward and Eddy (1950) note that of 35 strains of this virus isolated by them five produced a fatal pneumonia in mice after the first infection. In the work of Solov'yev, Marennikova and Gutman (1952) the strains of A-prime virus isolated in 1952 adapted more slowly to the bodies of mice than the 1949 strains did. In Table 49a corresponding data are presented on the adaptation process of the YeM strain (1949) and S5 (1952) strain.

The B-viruses adapt in a weaker manner to the bodies of mice. The well-known laboratory Lee strain became pathogenic for mice after a large number of passages in ferrets and white mice (Francis, 1940). Another strain of B-virus isolated during the same epidemic (TM) became pathogenic for mice after a few passages (Magill, 1940). Burnet was completely unable to adapt one of the strains of influenza virus to the bodies of mice (Burnet, 1947). The strains of D virus adapt poorly to the bodies of mice.

It has as yet been impossible to adapt the isolated strains to the bodies of mice.

The experiments mentioned were performed with the use of the method of intranasal passages. In certain cases it was possible to adapt the influenza viruses to other tissues. Thus, Stuart-Harris (1939), by means of cerebral passages of the A (WS) virus, obtained a neurotropic variant of this virus. Similar data have been obtained by Shakhnazarova and Sakharov (1946). Shen and Vanag (1954) obtained virus strains by the method of tumor passages which multiply well in tumor tissue.

The same inhomogeneity of the isolated strains may be encountered when passaging them in chick embryos. In general, the A viruses usually multiply well on the chorioallantoic membrane beginning with the first passage and accumulate in the allantoic fluid, whereas the A-prime and B viruses readily adapt to the amnion and only in subsequent passages do they accumulate in the allantoic fluid (Gorbunova, 1954). The C viruses multiply only in the amnion (Minuse and others, 1954).

Table 49a

Comparison of the Adaptation Process to Mice of Two Strains of Influenza A-Prime Virus

Number of Passage	YeM Strain (1949)				S5 (1952) Strain			
	Pathogenicity for Mice		Hemagglutination of Lung Suspension with Erythrocytes		Pathogenicity for Mice		Hemagglutination of Lung Suspension with Erythrocytes	
	Foci of Consolidation in the Lungs	Fatal Pneumonia	Guinea Pigs	Chickens	Foci of Consolidation in the Lungs	Fatal Pneumonia	Guinea Pigs	Chickens
1	0	0/10	0	0	0	0/4	0	0
2	0	0/10	0	0	0	0/4	0	0
3	±	2/5	0	0	0	0/4	0	0
4	++	3 10	40	10	0	0/4	0	0
5	+++	10/10	80	40	0	1/4	0	0
6	+++	10/10	80	40	0	2/4	0	0
7	+++	9/10	80	40	0	3/4	0	0
8	+++	10/10	160	80	0	5/5	0	0
9	+++	10/10	160	80	0	6/6	10	0
10	—	—	—	—	0	5/5	40	10
11	—	—	—	—	±	6/6	320	40
12	—	—	—	±	++	6/6	160	20

Key: Zero is a negative result; + the presence of foci of consolidation in the lungs; the numerator represents the number of animals which died from influenzal pneumonia; the denominator, the total number of animals in the experiment.

Certain of the isolated strains showed characteristic differences. Burnet (1936) obtained an endotheliotropic strain variant of A virus (Melbourne) by means of passages on the chorioallantoic membrane. Burnet and Lush (1938), in passaging the A (WS) virus in chick embryos, obtained a strain on the 26th passage which produced a hemorrhagic encephalitis in the chick embryo. Burnet and Bull (1944) obtained strains by means of prolonged allantoic passages of the A virus strains (Melbourne, WS) which produced a hemorrhagic encephalitis and focal pathology in chick embryos.

These observations show that the increase in the pathogenic properties during the course of passaging the influenza viruses is of an adaptive nature and is determined by the given organism in which the multiplication of the virus occurs.

In these investigations one fact attracts attention which was at first not given its proper importance: the increase of pathogenicity for the animal in which the passages are being made is usually accompanied by a loss of pathogenicity for other species of animals and for man.

Soviet investigators (Smorodintsev, 1936; Fal'kovich and others, 1949; Khayt, Vinogradova and others, 1942; Gamaleya, 1942) directed attention to this fact showing that the influenza viruses adapted to mice do not infect man or produce only a mild very transitory disease in him. Andrewes (1937), who unsuccessfully tried to infect people with the influenza virus adapted to ferrets, ran up against this fact. After the special experiments of Khayt, Vinogradova and Nartsissov (1949) performed in 1945 Zil'ber (1949) was the first to direct attention to this fact, pointing out the impossibility of obtaining good live vaccines from influenza virus strains adapted to various animals.

The characteristics of the pathogenic properties of the influenza viruses mentioned obtained a completely incorrect explanation in the foreign literature. In essence, all the statements published in the Anglo-American literature are variations of the Burnet conception which he presented in his book, "Virus as Organism" (1947). According to this conception, the development of pathogenic properties in influenza viruses during the course of passages in the bodies of susceptible animals is the result of spontaneous mutations and selection of pathogenic variants.

Sugg (1949) ran across the same phenomenon of multiplication of a non-pathogenic influenza virus strain in mouse lungs. The author considers the development of pathogenicity to be the result of selection of various viruses present in the original material. He considers variation of the original homogeneous population of the virus improbable.

Briody (1950) ascribes particular importance to the concept of the pathogenicity and capacity of being transmitted as innate properties of the virus particle. As a confirmation he presents the works of Hirst (1947) and Friedewald (1948) the authors of which observed an absence of pathogenicity in the first few passages in mice and hamsters of virus strains which had been isolated in chick embryos and interpreted the data obtained in accor-

dance with Burnet's conception.

Isaacs and Edney (1950) as well as Ada and Stone (1950) explain the causes and mechanism of variability of the influenza virus by the fact that the particles of the strain variant arose at some time or other as the result of mutations and since that time they have always been present in the Melbourne strain but can be manifested only under definite conditions, that is, in passages in maximum dilutions, at which time the concentration of the stronger basic (!) particles is reduced. The authors consider the Melbourne strain variant entirely non-pathogenic for mice, and they connect the mortality and pulmonary pathology with the effect of a small number of particles of the basic strain.

In this theory vitalism is obvious, because the authors regard the occurrence of variants as predetermined cyclic stages which start in a definite order (in a circle) and always return to the original form. The rules and regulations of the study of the pathogenic properties of influenza viruses during the course of their adaptation to the bodies of laboratory animals have been studied and understood by Soviet virologists. This problem has been studied in particular detail in recent years by Solov'yev and others (1949-1953).

Zhdanov (1951, 1953, 1954) and his colleagues (Gorbunova, 1953; Fadeyeva, 1952; Zakstel'skaya, 1952) have made and generalized on a number of investigations in this direction.

Solov'yev and Marennikova (1949) studied the process of adaptation of influenza virus to the bodies of mice. Strains of A-prime influenza viruses freshly isolated in chick embryos were passaged in mice, and during the course of the passages the pathogenic and other properties of the viruses were studied.

In Table 50 the results of experiments with the YeM strain are presented.

As is seen from Table 50, the multiplication of the freshly isolated virus culture in the tissues of the new host -- mouse lungs -- occurs very actively, which is shown without difficulty by means of such a sensitive quantitative method as injection of it into the allantoic cavity of the developing chick embryo. The process of virus multiplication is not associated with any apparent expression of its pathogenic properties, because in the first two serial passages the infected mice remained healthy. The pathogenicity of the virus for the new host was expressed only after the third passage, and it was not possible to establish any kind of essential quantitative changes in it compared with the first passages and original material. A

qualitative change in the virus occurred as a result of its adaptation to the mouse lung tissue cells. It occurred as a result of adaptation of the virus to new existential conditions. The hemagglutination reaction with a lung suspension of infected mice showed the presence of virus in the tissue beginning with the fourth passage, which in general coincided in time with the development of fatal pneumonias in the animals.

Table 50

Increase in the Pathogenic Properties of A-Prime Virus (Strain YeM) During the Course of its Passages in Mice

No of Experiment	Material For Investigation	Pathogenicity For Mice		Titration of Virus in Mouse Lungs in Embryos	Hemagglutination Reaction with Erythrocytes	
		Foci of Consolidation in Lungs	Fatal Pneumonia		Of Guinea Pigs	Of Chickens
1	Original Embryonic Culture	0	0/10	10-5	1/128	1/64
2	1st Passage in Mice	0	0/10	10-4	0	0
3	2nd Passage in Mice	0	0/10	10-5	0	0
4	3rd Passage in Mice	±	1/5	10-5	0	0
5	4th Passage in Mice	+	3/10	10-5	1/40	1/10
6	5th Passage in Mice	+	10/10	10-4	1/80	1/40
7	6th Passage in Mice	+	10/10	10-5	1/80	1/40

Note. The degree of pathogenicity of the virus for mice was determined by the presence of foci of consolidation in the lungs, for which an examination of the lungs was made 48 hours after infection in 15 mice (in each experiment).

Key. ± solitary foci of consolidation in the lungs of less than half of the mice; + presence of foci in more than half of the mice in the experiment. The numerator represents the number of mice which died over the course of 10 days of the observation; the denominator, the number of mice in the experiment.

After obtaining two variants of the YeM virus, one of which was not passaged any more after isolation (YeM-prime) and the other was adapted to mice (Ye-M-double prime), the authors studied their pathogenic properties for

various animals (Solov'yev and Marennikova, 1952). In experiments on white rats and gray rats a considerable increase was shown in the pathogenic properties: whereas the YeM-prime strain did not multiply in the lungs, did not produce disease, and brought about only a slight increase in the antibodies, the YeM-double prime strain multiplied actively in the lungs, producing a mild but clinically expressed disease, and led to the development of a definite immunity. These strains did not differ much in their pathogenicity and immunogenic properties with respect to swine.

In subsequent investigations (Marennikova, 1953) it was shown that the mouse-adapted strain possesses more marked toxic properties than does the original strain.

The authors (Solov'yev, Marennikova and Gutman, 1953; Solov'yev and Gutman, 1953) then compared the changes in the biological properties of the strain variants obtained from a single original strain (YeM-prime), by means of prolonged passage in mice (YeM-double-prime) and chick embryos (YeM-triple-prime). The mouse strain variant (YeM-double-prime) became highly pathogenic for mice after 102-130 passages producing a fatal pneumonia with an $LD_{50} = 10^{-5}$-10^{-6}, acquired a high degree of toxicity, causing the death of 77-80 percent of the mice after the intravenous administration of the virus: in rats immunized intranasally the antibody titer reached 1:1280-1:2560. At the same time, this strain did not increase its pathogenicity for chick embryos. In contrast to it the allantoic strain variant (YeM-triple-prime) which had under gone 50-60 passages in chick embryos began to produce the death of them regularly with an $LD_{50} = 10^{-7}$-10^{-8}, where as in comparison with the early passages the death of the embryos subsequently began to occur earlier (two to three days instead of five to six days). At the same time, this strain remained non-pathogenic and non-toxic for mice, and in rats immunized intranasally the antibody titer reached a total of 40-80. The antigenic structure of both strain variants (YeM-double-prime and YeM-triple-prime) remained identical to the original strain (YeM-prime)

Therefore, in these experiments it has been shown that the pathogenic properties are acquired during the course of adaptation of the virus to the body of the animal and that these properties are species specific, that is, directed against the species of animal to which the virus is adapted or against similar species (adaptation of the virus to mice increased its degree of pathogenicity not only for mice but also for the similar species, rats).

Data obtained by other authors confirm the correctness of these conclusions.

It would not be amiss to recall the experiments of Burnet (1936, 1937), on the basis of which this author drew many erroneous conclusions. Burnet observed the change in the pathogenic properties of the influenza virus when it was passaged in chick embryos.

In the first passages only slight pathology of the chorioallantoic membrane was observed; beginning with the 12th passage this pathology became more pronounced and had the nature of necrotic foci; after 63 passages a regular death of the embryos was noted with signs of hemorrhages in the brain and in the lungs. Along with the increase in the pathogenicity for chick embryos the pathogenic properties for ferrets and mice were reduced and then completely lost.

Chalkina (1943) passaged mouse strains of the virus in young white rats. During the course of these passages the virus adapted to the organisms of the white rats, producing a mild disease with diffuse but not very intense pathology in the respiratory tract and the formation of virus-neutralizing antibodies; however, afterwards the virus was exhausted. With the passage of the virus the pathogenicity for mice fell off and completely disappeared by the seventh passage.

In a later work of Luzyanina (1953) the influenza A (PR8) virus adapted poorly to the bodies of white rats after intranasal infection and was rapidly depleted in subsequent passages. In the experiments of Peterson (1954) this strain was adapted to the bodies of white rats with the intrapulmonary method of infection; thereby the influenza virus, while maintaining its antigenic structure, rapidly lost its pathogenic properties for mice.

Baydakova and Zil'ber (1948, 1949) studied the biological properties of the influenza virus during the course of its adaptation to the bodies of guinea pigs. During the course of its adaptation to the bodies of guinea pigs. During the course of the passages the original virus strain completely preserved its antigenic properties and conferred an asymptomatic infection on the guinea pigs. After 12 passages the virus did not become pathogenic for guinea pigs; however, it lost its pathogenic properties for mice.

Kravchenko and Sekretta (1950), repeating these experiments, showed that with a transition to passages in mice the virus rapidly regains its pathogenic properties.

Friedewald and Hook (1948) passaged A (PR8 and Ga47) and B (Lee) viruses in hamsters. The A viruses multiplied well in the lungs beginning with the first passages without producing pathology. In the seventh passage the virulence of the strains increased markedly; they began to produce pathology in the lungs; simultaneously their titer in

the lungs increased. The B virus proved to be virulent be ginning with the first few passages.

Mulder and others (1948) directed attention to the unusual behavior of the A virus strain isolated in ferrets (three passages) and then reinforced by chick embryo passages. After passages in chick embryos it was possible to adapt the strain to mice and produce a fatal pneumonia in them; when a variant of this strain was used which had been passaged a larger number of times in ferrets it did not adapt to the mice.

The process of adaptation and acquisition of pathogenic properties may be increased by the addition of certain substances to the infective material. Jones (1950) showed that the 1B45 B virus strain, which in ordinary intranasal passages does not adapt to the bodies of mice, can adapt and become pathogenic after the addition of a suspension of an H. influenzae, hemolytic streptococci, pneumococci, cholera vibrio cultures, chick erythrocytes, and certain bacterial extracts to the material being passaged.

Strains adapted to mice are characterized by a shorter lag-phase than allantoic strains and accumulate in higher titers (Davenport and Francis, 1951). The loss of toxicity after introduction into chick embryos of large doses of the allantoic strain is not observed in the corresponding experiments with mouse strains (Mak, Kri, 1951).

For the purpose of understanding the rules and regulations of the change in the pathogenic properties of viruses during the course of adaptation to the bodies of animals the investigations of Fadeyeva (1952) are of importance; she made a detailed study of the process of adaptation of influenza viruses to tissue cultures and to the bodies of mice.

The Shklyaver strain of virus A was used for the investigation, which after isolation and 24 passages in chick embryos afterwards underwent 10 passages in mice. This strain possessed a high degree of pathogenicity for mice, causing their death in high dilutions (LD_{50} = 10^{-6} -10^{-7}).

When the virus was transferred to a culture of lung tissue of a human embryo a marked decrease in the virus titer occurred; however, as early as after six to seven passages an adaptation of the virus occurred to the new conditions, which was expressed in an increase in the virus titer to 10^{-4} which afterwards did not increase (Fig. 101). It should be kept in mind here that in connection with the characteristics of titration of the virus in tissue cultures the actual value of the titer has to be increased by one or two logarithms so that after six to seven passages the virus titer in tissue cultures reaches 10^{-6},

and in the 17th-21st passage, 10^{-6}-10^{-7}.

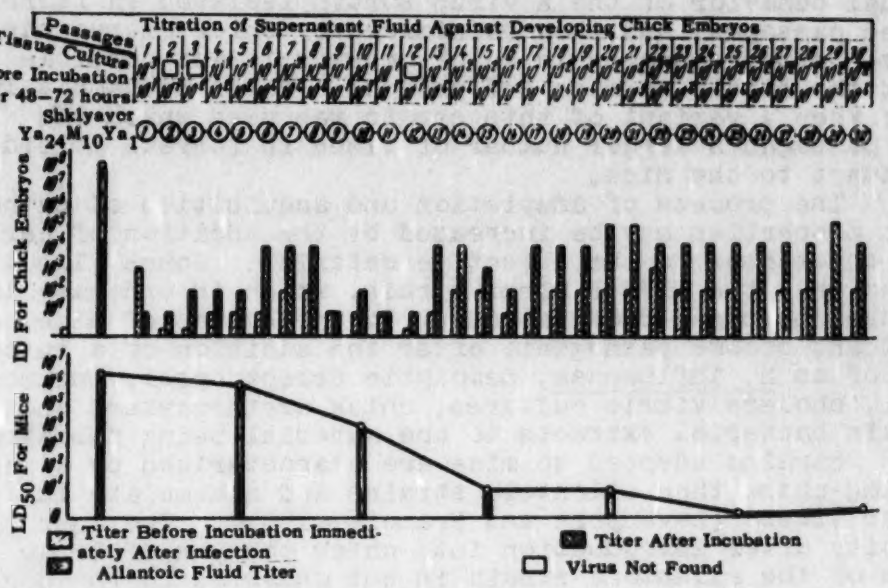

Fig. 101. Cultivation of Influenza A Virus (Shklyaver Strain) in Pulmonary Embryonic Human Tissue Outside the Organism (Fadeyeva, 1952).

In these experiments, however, it is important to note something else: with the passage of the virus in human tissue cultures its pathogenicity for mice gradually fell off. This process was completed more slowly than the process of adaptation to new tissue: the latter required a total of six to eight passages (the decrease in the virus titer between the ninth and the 12th passage was brought about by technical factors), whereas the pathogenicity for mice began to drop essentially after the 10th passage, and the strain completely lost its pathogenic properties for mice only in the 25th passage.

The adaptation process had a somewhat different course when this virus strain was passaged in mouse lung cultures (let us recall that the Shklyaver strain had undergone 10 passages in mice before the experiments). Here no decrease in the virus titer occurred, and beginning with the first passage it began to accumulate very actively

(10^{-4} or, calculating for comparable values, 10^{-6} respectively). This is natural, because the virus was transferred to mouse tissue from the bodies of mice (Fig. 102).

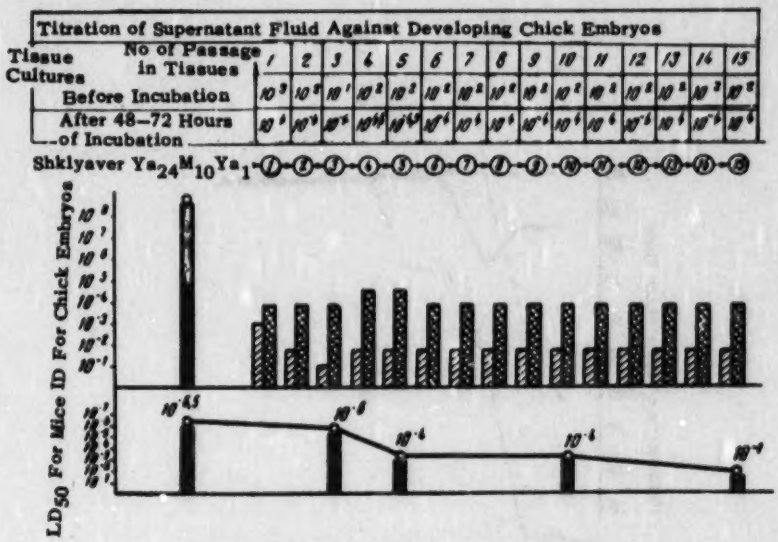

Fig. 102. Cultivation of Influenza A Virus (Shklyaver Strain) in Mouse Lung Embryonic Tissue Outside the Organism (Fadeyeva, 1952).

Key is the same as for Fig. 101.

Even in the passages in mouse tissues, however, a gradual decrease occurred in the pathogenic properties of the virus. With an original titer of $LD_{50} = 10^{-6.5}$ the pathogenicity fell of considerably in the 10th passage (10^{-4}), and in the 15th passage it became negligible (10^{-1}). Therefore, the properties of pathogenicity for mice acquired by the influenza virus during the course of its passages in mice are lost in passages not only in chick embryos or human tissue cultures but also in mouse tissue cultures.

Afterwards, a study was made of the readaptation process of the influenza virus to the bodies of mice (Fig. 103).

For the purpose of comparison four lines of the original Shklyaver strain (A virus) were taken which were passaged respectively in human tissue cultures, in a chick embryo, and in a mouse tissue culture. The criterion of com-

plete adaptation of the strain variants to the bodies of mice was the death of the latter after infection with the virus. For the purpose of adaptation to mice the strain variant which had been passaged in human tissues prior to this required six passages, whereas the strain passaged in mouse tissues became pathogenic for mice after three passages.

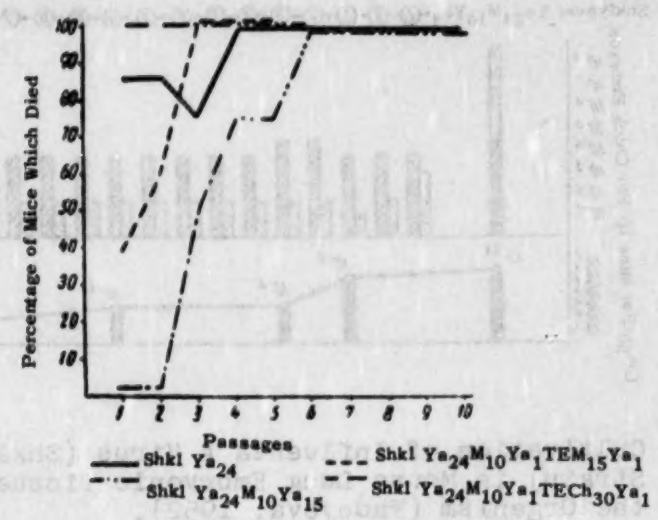

Fig. 103. Adaptation of Virus Strain Variants of Influenza (Shklyaver Strain) to the Bodies of White Mice (Fadeyeva, 1952).

Even more distinct results were obtained in experiments with the S6 strain (A-prime virus) where purer egg, human-tissue and mouse-tissue lines were prepared (Fig. 104). The strain variants passaged in human tissues and in chick embryos were non-pathogenic for mice during the first nine passages, and their pathogenicity began to be expressed and to increase between the ninth and the 14th-16th passages, after which they regularly caused the death of the animals.

The strain variant passaged in mouse tissues before this behaved differently. As early as in the fourth passage it began to cause the death of 75 percent of the mice, and in the seventh passage its pathogenic properties reached

a maximum.

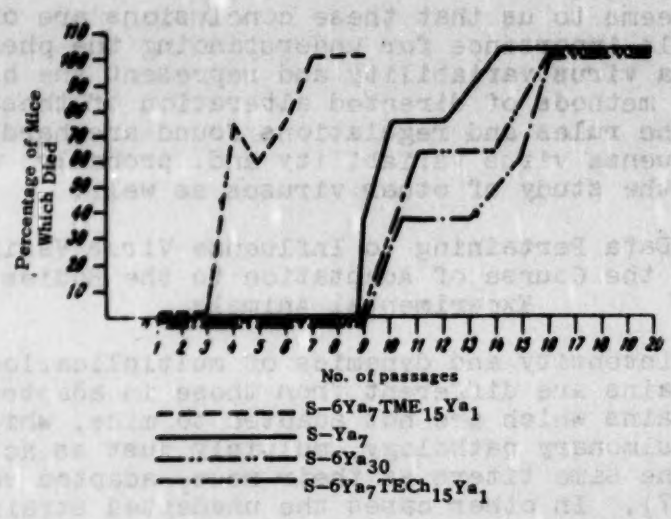

Fig. 104. Adaptation of Influenza A-Prime (S-6) Strain Variants to the Bodies of White Mice (Fadeyeva, 1952).

The investigations made permit us to draw certain general conclusions pertaining to the change in pathogenic properties of the influenza viruses.

1. The pathogenic properties of the influenza viruses, as has already been mentioned, are species-specific, that is, they are directed against the species of animals to which the virus has been adapted or is being adapted.

2. The acquisition of pathogenic properties by the influenza viruses is of an adaptive nature and directed at overcoming the protective forces of the body.

3. The pathogenic properties are elaborated in the process of overcoming the protective forces of the organism by the virus, and specifically, thereby, those of the intact organism. Therefore, in passages in tissue cultures the pathogenicity of the virus not only does not increase but rather falls off.

4. A preliminary adaptation to a tissue culture of

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a given biological species creates conditions for the more rapid transformation of the pathogenic properties during subsequent passages of the virus in the bodies of the same biologic species.

It seems to us that these conclusions are of more than a little importance for understanding the phenomena of influenza virus variability and represent the basis for working out methods of directed alteration of these properties. The rules and regulations found are hardly limited to influenza virus variability and, probably, can be applied to the study of other viruses as well.

36. Other Data Pertaining to Influenza Virus Variability During the Course of Adaptation to the Bodies of Experimental Animals

The intensity and dynamics of multiplication of unadapted strains are different from those in adapted strains. Certain strains which are not adapted to mice, while not producing pulmonary pathology, multiply just as actively and reach the same titers as their mouse-adapted variants (Hirst, 1947). In other cases the unadapted strains multiply more slowly, having a longer lag-phase and do not reach the maximum titers attained by the adapted strains (Davenport and Francis, 1951). Wang (1948) studied the infectious and hemagglutinating properties of the A-prime virus strain variants (Rhodes), one of which was adapted to chick embryos and the other to mice. The mouse strain variant accumulated notably in mouse lungs after 12 hours and reached its maximum titer ($10^{-7.5}$) after 24 hours. The egg strain variant multiplied more slowly and reached its maximum titer ($10^{-6.5}$) after 48-72 hours. The hemagglutinin titer (to chick erythrocytes) of the egg strain did not exceed 1:20; those of the mouse strain reached 1:1280. The author draws the inadequately grounded conclusion that the hemagglutinin titers are a measure of pathogenicity of the strain.

Sugg (1950) made a comparative study of certain biological properties of the same virus strain, one line of which had been adapted to chick embryos and the other, to mice. After the inoculation of small doses of the virus into mice intranasally the egg virus titer in the lungs after a day was equal to $10^{6.7}$; of the mouse virus, $10^{8.5}$. The author established the fact that the critical concentration of virus causing the death of mice was the same for both mouse lines: those infected with small virus doses died after it accumulated in the lungs in titers of more than 10^{8}; after infection with large doses, they died with titers of over $10^{6.6}$.

The investigations of our laboratory showed that the ideas presented above are only a schematic outline which needs definite correction. Zakstel'skaya (1952) established the fact that the hemagglutinating activity of the influenza virus ranges within broad limits during the course of adaptation of the virus to the bodies of experimental animals. After an infection with freshly isolated A-prime virus strains an asymptomatic infection develops in white mice with the accumulation of tremendous quantities of the virus (10^7-10^8 infective units) in the lungs; however, the hemagglutination reaction with lung emulsion is negative, and after immunization of rats with the lung virus no antihemagglutinins are formed or else are formed in negligible titers. When serial intranasal passages are made the virus becomes pathogenic for mice, first producing focal pathology and then a fatal pneumonia with hepatization of the lungs. At the same time as the development of pulmonary pathology the virus develops hemagglutinating activity also, and immunization with it now leads to the production of antihemagglutinins in rats in high titers. Therefore, the hemagglutinating activity of the influenza virus is closely associated with its pathogenic properties. At the same time, a massive accumulation of the virus in the body of an experimental animal may occur in the absence of any pathogenic or hemagglutinating properties.

Even earlier, Zakstel'skaya (1950) showed that toxic properties of the virus also occur during the course of adaptation of the influenza virus to the body of an experimental animal. The toxic factor acts on the body of the given biological species and is indivisible from the live virus particle.

The data presented supplement the material analyzed above concerning the adaptation process of viruses to the bodies of experimental animals and concerning the change in their biological properties during the course of this adaptation.

Jones (1950) studied the possibility of adaptation of the influenza virus to high temperatures. After a series of passages in tissue cultures with a periodic action of high temperatures on the virus the latter proved to be more thermostable, withstanding heating to 50° for 180 minutes; to 55°, for 45 minutes; and to 60° for five minutes, whereas the original PR8 strain died under these conditions.

The variability of the virus in the population was studied by Isaacs and Edney (1950). In using the method of maximum dilutions they isolated the MEL strain of the A virus from an allantoic culture; this was distinguished from the original strain by a receptor gradient, a lower

enzymic activity with respect to ovomucin and a lesser degree of pathogenicity for mice. In experiments with interference in chick embryos the variant was most sensitive than the original strain. The strain variant also had certain differences in its antigenic structure from the original as shown by the hemagglutination reaction: the degree of antigenic relatedness of the original and variant strains was equal to 1/4-1/8. Similar variants were obtained from the PR8 strain, but they could not be obtained from the Ocean Island and Lee strain.

Unfortunately, the further thorough investigations of population variability which were absolutely necessary here were replaced (Isaacs and Edney, 1950) by attempts to fit the facts observed into the framework of a conception of "spontaneous mutations," although the facts which they observed did not in any way fit within these artificial limits. Thus, in cultivating the original MEL strain (the authors arbitrarily designated it as M) a serum-resistant variant, MSr which preserved the strong original properties of the M strain was obtained in the embryos in the presence of antiserum. Despite the clearly adaptive nature of the variability with hereditary reinforcement of the acquired properties, the authors consider these changes the result of mutations (spontaneous). After passages of the variant of the original MEL strain with a lower enzymic activity (the authors designated it arbitrarily as M) in embryos with destroyed receptors an Mr variant was obtained with a more pronounced enzymic activity but which was somewhat different in the serological tests. Here, despite the clearly adaptive nature of the variation (adaptation to new conditions of being incorporated into a cell requiring changes in the nature of the enzymic activity) the authors consider it a mutation. It can only be considered unfortunate that such efforts were fruitlessly wasted in grounding a false conception which prevented the authors from correctly interpreting facts which exceedingly clearly illustrate the well known standpoints of Michurinist biology concerning the unity of the organism and the medium in which it is.

Certain characteristics of the behavior of influenza A-prime and B viruses in cultures of a homologous (that is, human) and heterologous (that is, chick) tissues were studied by Zakstel'skaya and Ritova (1954). The A-prime viruses adapt more rapidly to chick embryos, but in cultures of human tissues both viruses multiply effectively, whereby the concentration of virus in the tissue culture becomes quite considerable.

In comparing the growth conditions of both viruses it was made clear that the A-prime virus multiplies better

at 37°, when the tissue culture is growing actively, whereas the B virus multiplies better at 35°, when the tissue is growing poorly.

In concluding this section it is expedient to analyze Burnet's views, well known under the name of the theory (hypothesis) of O-D-variations. Burnet (1942, 1946, 1950) directed attention to the fact that freshly isolated influenza A virus strains differ from strains which have undergone a large number of passages in chick embryos in a number of biological properties. Strains freshly isolated by means of amniotic infection multiply more weakly in the allantoic cavity and immunize mice weakly, whereas strains cultivated for a long time multiply readily in the allantoic cavity and immunize mice well. He directed particular attention to the capacity of the influenza virus for agglutinating chick and guinea pig erythrocytes. Freshly isolated strains do not agglutinate chick erythrocytes so well and agglutinate guinea pig erythrocytes well; therefore, the ratio of the respective agglutination titers (C/P) is less than 0.25. After several passages, the virus begins to agglutinate chick erythrocytes well, and therefore the ratio C/P becomes greater than 0.5.

On the basis of these observations Burnet drew the conclusion that the influenza virus may exist in two phases, the starting, original phase (O) and the laboratory, derivative phase (D).

The investigations of Burnet mentioned were afterwards checked by numerous authors. Briody (1950) presents the following summarized data concerning the biological properties of O-D-variants of A viruses (Table 51).

On the basis of the C/P index and by analogy with A viruses Briody distinguishes two phases of the B virus: the natural phase (equivalent, E) and the derived phase (high-index, H).

Burnet's data on the O-D-variants and his views were subjected to experimental checking and criticism and proved to be incorrect. Many investigators occupied themselves in a complete study of the Burnet triad using his own material with the aim of establishing the possibility of applying it to differentiating newly isolated strains from laboratory strains.

Table 51

The Biological Properties of O-D-Variants of Influenza A Viruses (According to Briody, 1950)

No.	Properties	O–Phase	D–Phase
1	Rapidity of multiplication	+	++
2	Multiplication in the amnion	+	++
3	Multiplication in the allantois	—	+
4	Ratio C/P	0,05	0,5
5	Adsorption onto chick erythrocytes	—	+
6	The effect of 9.1 percent $CaCl_2$ on the ratio C/P	Decreases	No effect
7	Effect of one percent sodium citrate on the ratio C/P	Increases	No effect
8	Inhibition of hemagglutination by tears	—	
9	Pathogenicity for ferrets	—	+
10	Pathogenicity for guinea pigs	+	±

Hirst (1945, 1947) studied more than 40 strains of A virus isolated during the epidemics of 1941-1942 and 1943-1944, and 25 strains of the B virus isolated in 1945-1946. Through a special comparison of the efficacy of methods of isolating the virus in embryos from sick people Hirst noted cases of a direct isolation of the virus in the allantoic cavity, which contradict the views of Burnet, to the effect that the virus from man can be isolated only in the O form, that is, only in the amniotic cavity. In the experiments of Hirst new A strains readily adapted to mice; in addition, the ratio frequently became equal to unity during the first passages. At the same time, all the new strains of B virus did not show the agglutination characteristic of the O phase. Hirst believes that the significance of the O-D-phase variation phenomenon is not clear. The transition of the O form into the D form is based only on an increase in the agglutination titer of chick erythrocytes, since the adaptation to the allantoic cavity of the chick embryo and to the bodies of mice is readily accomplished with the direct isolation of the vi-

rus by these methods. It is interesting that in the series of passages in mice Hirst (1947) obtained a virus line with a very pronounced O-form of erythrocyte agglutination. Magill and Sugg (1948) occupied themselves with the problem of reversibility of the O-D-variants. The authors regarded the phenomenon of erythrocyte agglutination in its association with the physico-chemical state of the medium. At the same time, through the example of a very old laboratory strain they showed that virus cultures in vitro in the tissues of the chorioallantoic membrane produced a typical O-form of agglutination. With titration in a buffered solution of pH = 5.6 the D-form of agglutination was readily established.

Kalter and others (1948) isolated two strains of the A virus in Syracuse (Sy-prime and Sy-double-prime); one of these Sy-double-prime strains was highly pathogenic for mice despite its poor growth in the allantoic cavity.

Briody (1948) attempted to harmonize the Burnet theory with the contradictory data of other authors. He advanced the theory of phenocopies in place of the theory of mutants for explaining the transition of the O type of agglutination into the D type. The transition of the O phase into the D phase was established in vitro under the effects of heating, of solutions of various ions and of prolonged standing in the refrigerator. After confirming the results of Magill's and Sugg's experiment with the WS virus, Briody designated the O phase in the laboratory strain as "pseudo-O" or the Xo-phase.

The high agglutination titer of chick erythrocytes in newly recovered B strains could be brought to the same titer with guinea pig erythrocytes, so that in his experiments C/P = 1. In connection with this, he defines two phases, high, where $C/P < 1$. By analogy with the A virus Briody assumes that the pathogenicity for man is associated with the E phase.

Therefore, none of the investigators mentioned, including Burnet himself, could fit his observations made in the study of laboratory and newly isolated strains into the schema of O-D variability.

In a number of works, Andrewes and others (1944), Isaacs and Andrewes (1951) persistently deny the possibility of isolating new strains from man which would be pathogenic for mice without preliminary adaptation to the bodies of ferrets. All cases of isolation of strains primarily pathogenic for mice are identified by these authors with cases of laboratory contamination, that is, of passage of laboratory strains considered to be freshly isolated in the hands of workers who have no critical approach to research work.

In the problem of the apathogenicity of freshly isolated strains for mice the viewpoint of Andrewes is in complete agreement with the conception of Burnet, since the absence of pathogenicity for mice is one of the characteristics of the O phase of the virus.

In a number of works of authoritative investigators, both Soviet and foreign, however, there are definite indications as to the isolation of the virus from man directly in mice and of cases where pneumotropic strains and strains primarily pathogenic for mice have been isolated in embryos. With respect to this problem we refer to the works of Smorodintsev, Vlastelitsa and others (1950), Smorodintsev, Luzyanina and others (1952), Maksianovich and Il'yina (1951), van Rooyen and others (1949), Ward and Eddy (1950).

A particularly careful check was made in the works of Gorbunova and Marennikova (1949, 1950), Solov'yev and Marennikova (1950, 1952), Solov'yev, Marennikova and Gutman (1952), Gorbunova (1951, 1953), Gutman and Mentkevich (1954).

The facts of the changes in the biological properties of the influenza virus in themselves during the course of its isolation from the human body and adaptation to the organism of the developing chick embryo are quite understandable and to be expected. In this connection, Burnet's data do not add anything new in principle to the material presented above. Specifically, the increase of the capacity of the influenza virus for agglutinating chick erythrocytes after its adaptation to the chick embryo is also entirely expected, because we are dealing with an adaptation to parasitization in the chick organism and not with a different species of embryo.

At the same time, the criterion for determining the O or D phase in the form of the C/P index has been shown to be extremely unfortunate and chance.

As early as in the first work of Soviet authors (Gorbunova and Marennikova, 1949) it was shown that the ratio C/P does not define the quality of the strains isolated from man and cannot beused for the differentiation of them. As a matter of fact, experiments with the Gavr strain have shown that C/P, that is, the ratio of the number of the final virus dilution which agglutinates chick erythrocytes to the number of the final virus dilution which agglutinates guinea-pig erythrocytes was equal to 0.25 beginning with the fourth passage. This value (according to Burnet) corresponds to the index which characterizes the "human" virus strains. In the experiments the same index of the C/P virus was obtained adapted to mice and which multiplied abundantly in the allantoic cavity of the chick embryo. The greater sensitivity of guinea-pig

erythrocytes may be explained not so much by properties characteristic of various strains of influenza virus as by the species characteristic of the animal.

Afterwards, the study of new strains (Solov'yev, Marennikova and Gutman, 1952) gave the same results.

In testing the hemagglutinating effect of freshly isolated strains on chick and guinea-pig erythrocytes it was established that the index C/P for the LP/52 is equal to 2 and for the S5/52 strain, 0.5. In addition, in spite of Burnet's definition, freshly isolated strains showed the capacity of multiplying not only in the amniotic cavity of the chick embryo but also in the allantoic cavity.

Still more abundant material on this problem was collected by Gorbunova (1951, 1953).

The biological characterization of the newly isolated strains was made on the basis of a schema presented in the works of Burnet on the O and D variants of the influenza virus.

In Table 52 all the strains are listed with an indication of the data of their isolation, the average hemagglutination titers with chick erythrocytes and guinea-pig erythrocytes are presented, and the value C/P computed. The conditions of multiplication in the allantoic cavity are designated + (weak), + + + (abundant), - (no growth). Pathogenicity for mice is designated by the number of fatal doses of virus which produce a complete involvement of lung tissue (+ + +); infectivity with the presence of only focal pathology is noted by -+; no pathogenicity is noted by -.

The record of the titration experiments of the first five passages after isolation of the virus showed that the ratio C/P is not a constant characteristic for differentiating between freshly isolated strains and laboratory strains. This is seen particularly graphically in a number of the March 1949 strains, which are almost uniform with respect to all the other characteristics. Thus, two of them had a C/P = 1; in two, this ratio was equal to 0.8; in four, 0.5; and only in three, 0.25. Despite the difference in the ratio C/P (from 1-0.25), all the strains grew poorly in the allantoic cavity. The average hemagglutination titer in all 11 strains ranged within limits of from 1:40 to 1:10 with chick erythrocytes and remained equal to 1:40 with guinea pig erythrocytes.

These strains were weakly pathogenic for mice only in the first passage, acquiring a higher degree of pathogenicity after serial passaging.

The other strains of the A type, newly isolated in December 1948 and in January 1949, and the laboratory PR8 and WS had a C/P ratio equal to 1-0.8, multiplied well in

the allantoic cavity and were highly pathogenic for mice, whereby the two freshly isolated Shklyaver and Sher strains had even a higher virus titer in the first few passages (10^{-6}) than did the PR8 and WS laboratory strains.

Table 52

Characterization of Isolated Strains

Type	No of Strains	Time of Isolation	Average Reaction Titer With Erythrocytes		C/P	Growth in Allantoic Fluid	Pathogenicity For Mice
			Of Chickens	Of Guinea Pigs			
A	3	December 1948 r.	320	320	+	+++	+++ 10^{-6}
A	2	March 1949 »	40	40	+	+	+
A	2	1949 »	32	40	0,8	+	+
A	4	» 1949 »	20	40	0,5	+	+
A	3	» 1949 »	10	40	0,25	+	+
AWS	—	» 1933 »	260	240	1,08	+++	+++ 10^{-8}
A PR8	—	» 1934 »	256	320	0,8	+++	+++ 10^{-8}
B	2	December 1949 »	256	64	4	+	+++ 10^{-1}
B	2	» 1949 »	32	16	2	—	—
B Lee	—	» 1940 »	256	64	4	+	+++ 10^{-5}
B	2	« 1945 »	140	24	6	±	—
A of Swine		» 1931 »	256	320	0,8	+++	+++ 10^{-5}

We found a similar ratio of C/P equal to 0.8, a good growth in the allantoic cavity, and a high degree of pathogenicity for mice in the laboratory strain of the swine influenza virus.

In accordance with the data in the literature the ratio C/P was greater than unity in viruses of the B type in the experiments described: in freshly isolated strains this ratio was equal to four and two; in laboratory strains, four and six. The two freshly isolated strains are cultivated only by the amniotic route, they are not pathogenic for mice; the two other strains gradually adapt to the allantoic and are pathogenic for mice. Apparently, in isolating the virus from man the investigator ran up against strains with different adaptation capacities.

A series of special investigations was carried on for the study of variation of the O-D type in laboratory strains of the influenza virus. With this aim in view, strains of influenza viruses were cultivated in chick embryos by different methods. In the analysis of 11 strains

it was possible to observe three different types of variability of the A-A-prime viruses in accordance with variations in the ratio C/P (Table 53).

Table 53

Types of Hemagglutination Reaction According to the C/P Feature of Newly Isolated Strains in the First Few Passages in Embryos

Name of Strain and Date of Isolation	Passages in Embryos							
	II		III		IV		V	
	Titer	C/P	Титр	C/P	Titer	C/P	Titer	C/P
Sdb 18 March 1949	10/40	0,25	32/01	0,5	20/40	0,5	40/40	1,0
Lshcho 18 March 1949	8/32	0,25	20/80	0,25	20/40	0,5	40/40	1,0
Yaks 22March 1949	32/64	0,5	32/64	0,5	40/40	1,0	20/20	1,0
Klim 2/IV 1949	8/32	0,25	8/64	0,12	20/40	0,5	40/40	1,0
On the a verage		0,3		0,3		0,6		1,0
S-6 14/III 1952	32/64	0,5	32/128	0,25	16/128	0,12	20/80	0,25
YeM 1/IV 1952	10/20	0,5	16/64	0,25	16/32	0,5	16/32	0,5
Chizh 1/IV 1952	4/32	0,12	8/24	0,3	10/30	0,3	10/30	0,3
Ku 2/IV 1952	8/16	0,5	18/56	0,3	20/40	0,5	20/40	0,5
On the average		0,4		0,25		0,3		0,4
S-3 8/III 1952	4/16	0,25	240/80	3,0	40/80	0,5	80/80	1,0
Gavr 18/III 1952	4/4	1,0	10/40	0,25	16/16	1,0	10/20	0,5
Bel 30/III 1952	32/16	2,0	80/80	1,0	20/20	1,0	20/80	0,25
On the average		1,1		,4		0,8		0,6

Key: In the column "Titer" the numerator of the fraction designates the titer in the hemagglutination reaction with chick erythrocytes; the denominator, with guinea pig erythrocytes. In the column C/P the decimal indicates the ratio of titers the absolute value of which is given in the preceding column.

As is seen from Table 53, in some cases the variation occurred according to the O-D type; in other cases, the virus remained for a long time in the O phase; in still other cases, the reverse type of variation was observed,

that is, D-O.

In Figs. 105-107 all three types of variation of the strains studied are presented; in Fig. 108, these types of variations are given in a schematic form.

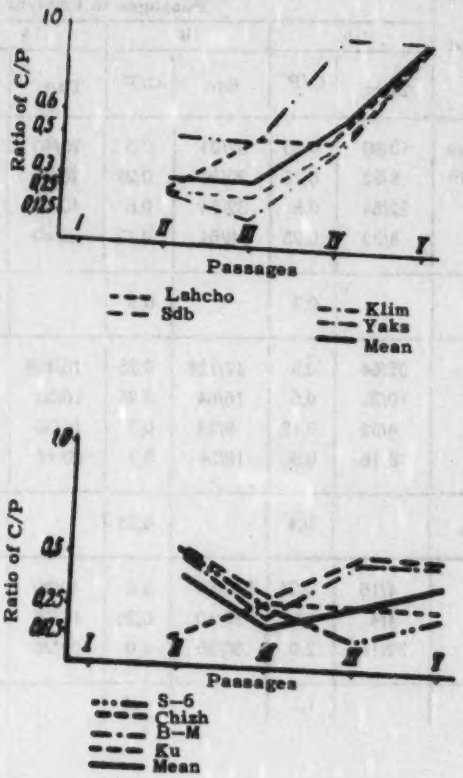

Fig. 105. First Type of Variation of the O-D C/P Characteristic (Gorbunova, 1953).

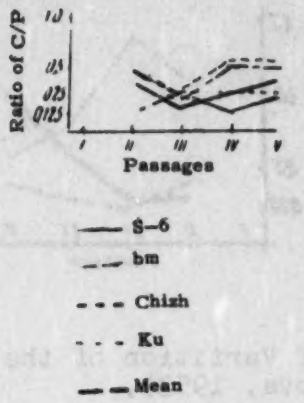

Fig. 106. Second Type of Variability of the C/P Characteristic -- Prolonged O Phase (Gorbunova, 1956).

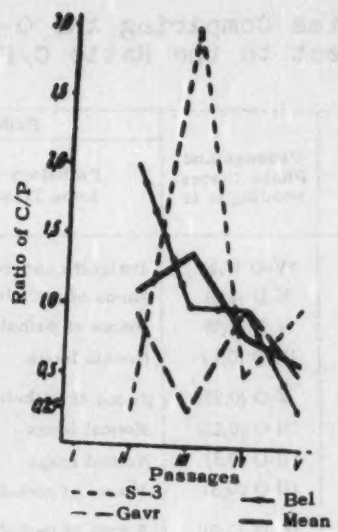

Fig. 107. Third Type of Variation According to the Characteristic C/P; O D (Gorbunova, 1956).

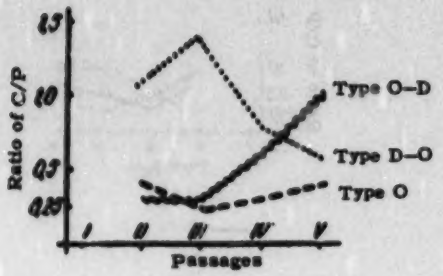

Fig. 108. Types of Variation of the C/P Characteristic (Gorbunova, 1953).

It was impossible to find any association between the O-D state of the virus according to Burnet's criterion and its pathogenicity for mice, which is demonstrated distinctly by the data presented in Table 54.

Table 54

Pathogenicity for Mice Comparing the O- or D-Phase With Respect to the Ratio C/P

Strain	Phase with Respect to C/P	Passage and Phase Corresponding to it	Pathogenicity for Mice	
			Pathology of Lung Tissue	Final Dilutions Producing Pathology of Pulmonary Tissue
Sdb	O–D	IV-D (0,5)	Insignificant focus	Undiluted
Lshcho	O–D	V-D (1,0)	Focus of pathology	10^{-2}
Yaks	O–D	V-D (1,0)	Focus of pathology	10^{-1}
Klim	O–D	III-O (0,12)	Normal lungs	—
S–6	O	V-O (0,25)	Focus of pathology	10^{-2}
YeM	O	III-O (0,25)	Normal lungs	—
Chizh	O	III-O (0,3)	Normal lungs	—
Ku	O	III-O (0,3)	Focus of pathology	10^{-1}
S–3	D–O	III-D (3,0)	Focus of pathology	10^{-2}
Gavr	D–O	V-D (0,5)	Focus of pathology	10^{-3}
Bel	D–O	III-D (1,0)	Insignificant focus	Undiluted

Therefore, Burnet's factual material concerning the significance of the C/P index as a criterion for determining the O- and D-phases of the influenza virus have not been confirmed.

Burnet's attempt to analyze the facts which he observed is completely inacceptable. Despite the clear-cut production of a change in the biological properties of the influenza virus by the influence of a medium which is new for them (chick embryo) and the adaptive nature of these changes, Burnet (1949), in spite of the facts, treats them as a simple selection of mutants. In another place (1946) he asserts directly that these changes are not brought about by any influence of tissues of the new host.

The variability of viruses, in spite of the opinion of Burnet, depend entirely on the influence of external conditions of the environment, which for these intracellular parasites represent tissue cells of the new host. This process does not necessarily occur only through selective survival of the mutants. Burnet's idea that "a single particle precedes the thousands and millions of influenza virus particles formed from the multiplication of O-variants and is found in the D form" is worthless. The results of our experiments show the reverse: the adaptation of the virus to the organism of the new host occurs without any essential quantitative transformations, and a qualitative change in the properties of it occurs under the influence of the tissues of the new host during the course of adaptation to the new existential conditions. The new property of the virus -- its capacity of producing a fatal pneumonia in mice -- is expressed after the third or fourth successive passage, whereas its titers in the tissues of the lungs remain high throughout all the experiments. Therefore, the occurrence of pathological signs is dependent not only on the degree of multiplication of the virus but chiefly on the qualitatively new properties of pathogenicity for a given species of animal acquired as a result of adaptation to new existential conditions.

37. Variability of Influenza Viruses and Evolution of Them

We have analyzed the main facts concerning the variability of influenza viruses in the epidemic process and in the laboratory experiment, directing special attention to a change in the pathogenic properties, immunogenicity and antigenic structure as the most essential properties of the influenza viruses. A tremendous number of facts which have accumulated at the present time show with perfect evidence that the changes in the biological proper-

ties of the influenza virus are strictly determined by and adequate to the changes in the environmental conditions and primarily to the effect of the human body, the bodies of experimental animals and tissue cultures in which the activity of the influenza virus is occurring. Attempts to understand these acts from the standpoint of Weismannism-Morganism of different shades (the theories of O-D-variations, P-Q variations and gene exchange) have been shown to be completely worthless. The application of the evolutionary theory of Darwin-Michurin has made it possible not only to understand the facts observed but also to control the variability phenomena of the influenza viruses. It seems expedient, in conclusion, to analyze the roots of variability of the influenza viruses and the problems of their evolution.

From this point of view certain theories deserve analysis which pertain to general problems of variability and evolution of influenza viruses (Andrewes, Burnet, Fal'-kovich).

In one of his works, Andrewes (1942) has presented certain considerations concerning the routes of variation of the influenza viruses and of their evolution. He correctly points out the dependence of changes in the antigenic structure of influenza viruses on the specific immunity of the population to them. Unfortunately, this correct idea is swamped in a sea of speculative reasoning and erroneous inferences. Andrewes advances the idea of the existence of a "basic virus" which is incapable of producing the disease in man. This virus then goes through six stages of increase in its virulence, becoming "epidemic" and a "pandemic" virus. Andrewes measures the degree of virulence (virulence generally not for man!) by the capacity of involving ferrets and mice. Something in the nature of a cyclic development on the type of the R-S-R cycle in bacterial forms is obtained. Here, everything is incorrect; everything is turned topsy-turvy. Instead of interpreting the influence of the epidemic process on the variability of the influenza virus the variability itself is revealed as the cause of the epidemic process -- a throwback to an old theory attempting to reduce the complex epidemic process to just a single microbial factor. Virulence is treated by the author as some kind of abstract concept unrelated to the organism against which it is directed. The standpoint that "the basic virus" can be converted either into an A virus or into a B virus is completely speculative. The entire variability of influenza viruses is regarded as movement around a tight circle rather than as a progressive process or development. The idea of the existence of a large number of influenza viruses aside from

those known has very little basis. Therefore, Andrewes' hypothesis is fantastic and erroneous.

Burnet (1946) in his monograph "Virus as Organism" devotes a special chapter to influenza. Certain general considerations which he expressed in this book deserve attention. Among them are, for example, the standpoint that infectious diseases should be studied primarily as an ecological problem of interaction between two species of organisms. The assertion that influenza A and influenza B are two different infectious diseases is justifiable.

Unfortunately, much of the author's reasoning is based on speculative analogies or not very reliable data. Thus, for example, based on the hypothesis of the origin of the majority of epidemic diseases of mankind from similar animal diseases, Burnet draws the conclusion that swine constitute the reservoir of the influenza virus, although afterwards he makes the reservation that any attempts to establish the history of the influenza virus evolution are premature. This, however, does not keep him from continuing to construct his analogy and expressing the idea of the latent carriage of the influenza virus by man and of the liberation of the latter from cells under the influence of nonspecific infection or an external stimulus. In discussing further the theory of O-D-variations which he propounded he reduces all the phenomena of influenza virus variability to the survival and spread of the fittest mutants and even considers that a single such mutant occurs spontaneously in every million particles of influenza virus. The factual side of this problem and the methodological error of Burnet's conception were analyzed above. Nor did he succeed in making any generalizations concerning the lines of variability of the influenza viruses although he had promised that he would at the beginning of the chapter. A preconceived notion of the possibility of occurrence of dangerous mutants stimulated him a priori to reject the method of immunization against influenza through the respiratory passages using live vaccine virus which he had suggested.

The views of Fal'kovich concerning the interrelationship of the influenza virus and the Bacillus influenzae of Afanas'yev-Pfeiffer are constituted by a distinct recurrence of pleomorphism. In a number of works Fal'kovich and other workers in the Moscow Institute imeni Mechnikov (Fal'kovich and Yanushevich, 1947; Fal'kovich, 1947; Ginzburg, 1947) show that the Bacillus influenzae has much in common with the virus of influenza. In experiments on mice they showed a corresponding effect of the Bacillus influenzae on the development of influenzal pneumonia. The sera of animals immunized with Bacillus influenzae produced

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neutralization and complement-fixation with the influenza virus. After subjecting the Bacillus influenzae to freezing and thawing or to grinding of washings containing it with agar, Fal'kovich infected mice with this material and produced a chronic pneumonia which was transmitted in subsequent passages. The basic idea of this work is the not always distinctly expressed idea that the influenza virus and the Bacillus influenzae are stages in development of the same pathogen. Otherwise, it would be difficult to understand the hazy expression "interrelationship," which, as the director of all these works, Krestovnikova interprets specifically in this sense (Krestovnikova, 1947).

On familiarization with the works mentioned there is much that remains unclear. The authors speak of "the interrelationship" between the influenza virus and the Bacillus influenzae, basing their conclusions on the similarity of the antigenic structure of both causal organisms. At the time of publication of these works, however, there were at least two causal organisms of influenza rather than one. The authors worked with the A virus (M_{13} strain). And what about the B virus? Where is its analogue among the bacteria? It is not surprising, therefore, that on checking her data Fal'kovich did not confirm the facts which she described. It was shown that lysates of the Bacillus influenzae absorb the influenza virus onto themselves, which latter exists in the air and on articles in the rooms in which the experiments were being performed with infection of mice with the influenza virus (Kravchenko and Sekretta, 1949). What the authors mentioned did not say completely (Krestovnikova, Fal'kovich) was expressed in its full form by Bosh'yan (1949), who reduced the idea of the identity of viruses and bacteria to the absurd. The sorry results of his "research" are well known. After noting the "interrelationship" indicated between the ideas of Bosh'yan and the works of the authors mentioned above (Krestovnikova, Fal'kovich), we would only like to mention the impossibility of a rebirth of the pleomorphism of the 19th century. Any attempts at reviving it signify a voluntary or involuntary tendency toward ignoring many decades of persistent work by microbiologists and virologists.

Therefore, none of the three theories mentioned gives us the proper explanation of the problems of influenza virus variability and evolution. These problems may obtain their explanation through a comparison of the data of influenza virus variation with the main characteristics of the pathogenesis and epidemiology of this infection.

Above, the main trends in the variation of the influenza viruses have been analyzed, which are found in the study of the epidemic process and in the laboratory experi-

ments. It has been shown that in the course of the epidemic process a continuous change in the antigenic structure of the influenza viruses occurs under the influence of specific immunity to it which develops in the population. It has also been elucidated that entrance of the virus into the body of another biological species does not affect the antigenic structure of the virus but changes its immunogenic properties. Finally, it was established that the pathogenic properties are elaborated by the virus in the course of overcoming the protective forces of the organism. In Fig. 109 these processes of influenza virus variation are represented schematically.

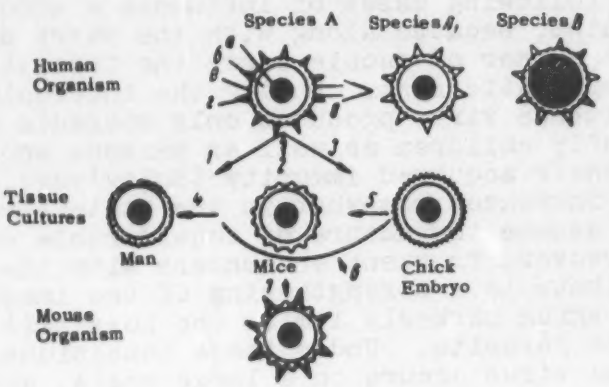

Fig. 109. Diagram of Variation Processes of Influenza Viruses.

a -- group-specific antigen, common to all influenza viruses; b -- species-specific antigen (A, A-prime and B); c -- immunogen, the specificity of which depends on the host organism tissues; d -- virulence factor. The arrows show the direction of variation of influenza viruses: 1, 2, 3, 4, 5-6 -- variation as the result of adaptation to the tissues of various species of animals with which the immunogenicity of the strains is associated; 7 -- variation as the result of adaptation to an animal organism with which the virulence of the strains for the given species

of animal is associated; 8 -- variation under the influence of specific immunity factors leading to a change in the species-specific antigen and to the formation of a new species (Zhdanov, 1953).

It seems expedient to attempt to give an explanation of the influenza virus variation processes observed, which, in connection with a considerable deepening of our knowledge concerning this infection, seems to us entirely possible.

The high degree of infectivity of an influenza patient, the air-droplet route of transmission and the short incubation period usually bring about a stormy development of cases of influenza and the inclusion of considerable territories and population groups. As our investigations have shown, following cases of influenza a strong immune segment remains, because along with the overt diseases a considerable number of people carry the infection in a latent or asymptomatic form. During the interepidemic period the influenza virus produces only sporadic cases, involving chiefly children as well as persons who have gradually lost their acquired immunity (Solov'yev). The morbidity rate increases somewhat in the wintertime; however, it does not assume the nature of considerable epidemic outbreaks. Moreover, frequent encounters with the influenza virus contribute to a strengthening of the immunity in the population, which markedly limits the possibility of circulation of the parasite. Under these conditions, death of the influenza virus occurs on a large scale, and it cannot overcome the collective immunity of the population, because after coming into contact with the mucous membranes of immune persons the virus dies out rapidly. Apparently, the ubiquitous disappearance of the A virus was one of the results of the process described. This influenza virus at the present time has been preserved only in laboratories, being, incidentally, attenuated as a result of passages in laboratory animals.

The high degree of adaptive variability of the influenza pathogen, however, like that of other viruses, makes possible the occurrence of new varieties of it. Repeated passages through an immune organism lead to the occurrence of new virus varieties and primarily to varieties showing immunological differences; as a result, such viruses overcome the immunity which has been built up by the population.

The variation of the influenza viruses under natural conditions proceeds specifically in this direction also, as a study of strains isolated in various countries at various times has shown. The quantitative changes built up

culminate in the appearance of new viruses which show marked immunological differences from the ancestral form. Under certain conditions, these viruses can acquire a universal distribution. An example of such a process is the formation of the A-prime virus, which, after its origin several years ago, spread everywhere in a short period of time and became the predominant influenza virus species. Probably, such a process is occur ing at the present time (1957) in connection with the pandemic of "atypical" influenza.

The common origin of influenza viruses is indubitable, which is confirmed not only by their far-reaching biological similarity but also their serological relatedness. In antigenic structure, the influenza A, A-prime and swine influenza viruses are most similar; however, the influenza viruses A, B, C and D also converge in the fact that they parasitize the human body and produce diseases which are not very easily distinguishable by their clinical characteristics. There is no doubt that the human influenza viruses have a common origin and a direct genetic relationship. However, at the current level of our knowledge it is hard to express any sound considerations of the roots of origin of the influenza viruses and of the early stages of their evolution. The origin of swine influenza viruses may be imagined in a more definite manner.

This disease attracted attention in 1918, at which time during an influenza pandemic in the United States an epizootic of swine developed which was similar in its clinical course. The virus which was afterwards discovered by Shope (1931) proved to be very similar (although not identical) in its antigenic structure and other biological properties with the influenza virus which was isolated from people two years later (Smith, Andrewes and Laidlaw, 1933).

Like other respiratory infections of animals swine influenza spread through contact of healthy animals with sick ones. Apparently, not only sick animals but also healthy virus-carriers may be the sources of the infection. Swine influenza, in addition to the United States, was found in many countries of Europe and Asia, including the USSR (Revo, 1954).

In the 1940's the unusual features of the results of work obtained by Shope on the epizootology of swine influenza attracted attention.

Shope (1941, 1943) injected H. influenzae suis intramuscularly and produced virus influenza in apparently healthy animals. These experiments gave him the grounds for the supposition that healthy and susceptible animals may carry the virus. In his search for the reservoir of the virus he directed attention to the presence of lungworms in the animals, namely, Metastrongylus elongatus and

Choerostrongylus pudendotectus. Experiments were performed in which the hogs were preliminarily (three weeks to three months before) fed the lungworms from swine which had been sick with influenza, after which at different intervals they were injected intramuscularly with cultures of H. influenzae suis. After the three injections the swine became ill with influenza; virus-neutralizing antibodies appeared in their blood after having had the infection. Similar results were obtained in experiments with earthworms, Eisenia Foetida, Allolobophora caliginosa, A. longa and Lumbricus terrestris. On the basis of these experiments the author came to the conclusion that the lungworms of swine are vectors and intermediate hosts of the swine influenza virus, and injection with H. influenzae suis is a factor provoking the development of the infection.

In these experiments there was much that remained unclear, and they were not repeated by other investigators or by the author himself. Epizootological practice has not as yet confirmed these data and rather indicate the contact route of infection of swine from one another and of an ordinary acute course of the disease in animals more like human influenza.

For the purpose of understanding the origin of the swine influenza virus three groups of observations are of great importance.

First, the high degree of susceptibility of swine to human influenza has been solidly established. In addition to the data presented in the preceding chapter it is fitting to present the observations of Kono and coauthors (1951), Solov'yev and Marennikova (1952), indicating the high degree of susceptibility of swine to influenza A-prime virus.

Secondly, in a number of cases it has been established that the etiologic structure of swine influenza is a kind of reflection of the etiologic structure of influenza in people. Above it was mentioned that the Shope virus of swine influenza isolated in 1931 is very similar in its antigenic structure with the viruses of human influenza isolated in 1933. Smith, Andrewes and Laidlaw, Francis, Quilligan and Minuse (1949) observed an epizootic of swine in Korea. The virus isolated from the swine (Oti strain) proved to be similar in its antigenic structure to the influenza A-prime virus. Young and Underwahl (1951) investigated 1,723 hog sera for their content of antibodies against influenza viruses. In swine which had been sick with influenza in 1949-1950 antibodies predominated against the swine influenza virus and the WS strain. In solitary cases sera were encountered which contained antibodies against the PR8 and Lee viruses. In 1950-1951 antibodies

against the PR8 and Lee viruses were encountered much more often. These observations established the possibility of infection of swine with influenza B virus. Gompels (1953) made a comparative study of the antigenic structure of six strains of swine influenza virus isolated in England, Ireland and the United States with influenza viruses of people (WS, PR8, FM1). The American strains which had been isolated from 1931 through 1949 (four strains) were identical with one another (among them was the Shope strain, 1931) and were different from the influenza viruses. The English strain isolated in 1941 produced weak cross reactions with the American strains and the influenza WS virus. The Irish strain had no antigens in common with the American strain and had a slight antigenic relationship to the English strain. These data were obtained in cross immunization reactions. In performing the complement-fixation reactions with soluble antigens all the swine and human viruses were found to be similar to the A group, having common antigens.

The third group of facts pertain to the occurrence of epizootics of swine influenza in connection with influenza epidemics in people. It has been mentioned above that the occurrence of swine influenza epizootics in the United States in 1918 coincided with pandemics of human influenza. Unfortunately, in the work mentioned of Francis, Quilligan and Minuse, who were the first to establish cases in swine produced by a virus similar to the A-prime virus, the circumstances of occurrence of the epizootic were not clarified. Therefore, the observations of Dreguss (1943) wherein the great probability of infection of swine from people is established of considerable interest. In February 1941 in Hungary there was an epidemic of influenza which began in Budapest. Simultaneously, an epizootic of swine influenza occurred in the suburbs. In the investigation of the sera of the swine which recovered no antibodies were found in them against the museum strain of swine influenza virus, but, on the other hand, high titers of antibodies were found against the museum strain of the influenza A virus (up to 1:100) and even higher titers (1:250) against the influenza A virus which had been isolated from people in Budapest during this epidemic of influenza.

All these data permit us to make deductions to the effect that swine influenza is the result of adaptation of the human influenza virus to the swine organism. The facts presented above confirm this supposition, which we have expressed previously (Zhdanov, 1953) and permit us to establish certain characteristics of swine influenza. In the work by Gompels presented above the fact of the invariability of the antigenic structure of swine influenza viruses

isolated in the United States from 1931 through 1949 attracts attention. These viruses are in their antigenic structure most similar to the influenza A virus (WS). Nevertheless, the etiologic structure of human influenza viruses during these years in the United States of America underwent a notable evolution. As early as 1934 the PR8 virus had been isolated there, which was notably different from the WS virus in its antigenic structure. Beginning with 1945 A-prime viruses gradually began to appear which, beginning with 1947, became predominant. Simultaneous with this, the A viruses stopped circulating among the population.

These facts become understandable if we keep in mind the fact that adaptation of influenza viruses to the bodies of other biological species does not lead to any kind of essential change in their antigenic structure. The nature of the epidemic influenza process is completely different from the epizootic process of swine influenza. In the former case a circulation of the pathogen occurs among a considerable part of the people having various degrees of specific immunity, producing a sequential change in the antigenic structure of the virus. In the latter case, the virus almost always comes into contact with the non-immune organism (let us recall that swine after reaching the age of one or two years are killed for food) and, therefore, is not subjected to the effect of specific immunity on its antigenic structure. Therefore, swine constitute a distinctive reservoir of human influenza viruses the antigenic structure of which is preserved unchanged until continuity of the epizootic process is assured, making the independent existence of the viruses possible in the swine population. Probably, by far not every large-scale influenza epidemic among people is associated with an infection of the swine stock. A local epizootic of swine influenza caused by infection of them with human influenza rather infrequently spreads over considerable territory and leads to the establishment of this virus among swine. This is probably explained by the comparatively small group of influenza viruses circulating among the swine. This also gives us the grounds for supposing that the best studied swine influenza viruses (Shope strain and strains similar to it which have been isolated in the United States and other countries) became pathogens of the disease in swine much earlier than the first human influenza viruses were isolated. The supposition is very probable that these viruses separated from man and adapted themselves to swine during the influenza pandemic of 1918-1920 as the result of the epidemic spread of the disease among people during these years and perhaps also in association with the presence of

a broader pathogenicity spectrum and more pronounced virulent properties in these viruses.

If these data are confirmed it may be supposed that the viruses of swine influenza are the distinctive indicators of definite stages in the evolution of influenza viruses, and the Shope virus of swine influenza has possibly preserved the antigenic structure of the pathogen of Spanish influenza of 1918 which has long ago disappeared and the descendants of which are the modern influenza viruses.

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FOURTH CHAPTER

PATHOGENESIS

38. General Characterization

A general characterization of the pathogenesis of influenza on the basis of current data is represented in the following form.

The influenza virus enters the upper respiratory tract with the air and settles here on the surface of the mucous membrane of the nasopharynx. Owing to the presence of the hemagglutinin the virus is adsorbed onto the surface of the cells and then penetrates into them and multiplies intracellularly. Multiplication of the virus in the mucous membrane leads to necrosis and desquamation of the cylindrical epithelium and simultaneously exerts a more traumatic effect on the body. The latter depends both on the effect of the virus at the site of its multiplication and on the entrance of the virus and of the products of its activity into the blood.

The most important results of virus multiplication in the body, determining the pathogenesis of influenza, are the nervous system depression, the suppression of the protective functions of the body and local pathological changes. As a result of the decrease in the resistance of the body secondary infection associated with multiplication of the conditionally pathogenic microflora of the respiratory tract is of importance in the pathogenesis of influenza.

Multiplication of the virus and the development of the pathological processes produced by it cause a reaction in the organism -- the development of a specific immunity and the recovery from pathology produced in the body by the virus and by the conditionally pathogenic microflora. All these processes proceed in an acute manner and develop in a short time, in connection with which the disease itself is brief, and recovery occurs quickly.

The spread of the influenza virus in the respiratory passages occurs chiefly per continuitatem. When liberated from affected cells the virus involves new areas of respiratory epithelium, dropping down from the upper parts of

the respiratory tract along the bronchi to the smallest bronchioles and alveoli. Along with the respiratory discharge the influenza virus is ejected into the air in the form of very small droplets and larger pieces of sputum on coughing, sneezing and talking.

The toxic effect of the virus, which is manifested differently (general and local effect), is of importance in the pathogenesis of influenza. Apparently, many of the manifestations of influenza which are characterized by clinicians as intoxication are associated specifically with this property of the influenza virus.

Naturally, the brief description of the pathogenesis of influenza presented is schematic and applies to cases with a disease of moderate severity and uncomplicated. As a matter of fact, the infectious process in influenza is more complex and varied. Depending on the condition of the body the disease may assume a milder or a more severe course.

With a mild course of the disease the general and local pathological phenomena are less pronounced and rapidly compensated by the body up to the point where the infection may have an asymptomatic course. In severe cases the general and local pathology are pronounced; various components of the pathologic process may become prominent: nervous system depression (toxic influenza), tissue pathology at the site of multiplication of the virus (primary influenzal pneumonia), suppression of the protective functions of the body (development of complications produced by conditionally pathogenic flora).

It seems expedient, therefore, to discuss the various aspects of the pathogenesis of influenza in greater detail.

39. Reaction of the Influenza Virus With Susceptible Tissues. Multiplication in the Chick Embryo

For the purpose of understanding the pathogenesis of influenzal infection investigations made in recent years directed at the clarification of the reaction of the influenza virus with susceptible tissues are of importance. Much has become clear in the mechanism of this reaction after the study of the hemagglutination reaction, more accurately, the reaction of the virus with erythrocytes, because it was shown that the reaction of the virus with susceptible tissues of the respiratory tract has the same characteristic features, at least in the initial stages of the infection.

The general picture of multiplication of influenza viruses in the chick embryo is represented in the following form.

After infection is introduced into the allantoic sac both a local and a generalized process of virus multiplication develop. The limitation of absorptive processes from the allantoic cavity contributes to the localization of the infection inside the allantoic sac. With a normal function of the allantois its contents are supplemented by the embryonic waste products. Physiological absorption of the contents of the allantoic sac is apparently of a localized nature.

It may be assumed that generalization of infection occurs in pathology of the tissues of the allantoic sac in which its normal function is disturbed. Then, instead of keeping the metabolic products from contact with the fetus or organs supplying it with food and oxygen, the allantoic cavity is converted into the cause of death of the embryo owing to a pathological absorption of its contents.

A small number of virus particles introduced into the allantoic cavity infects solitary cells of the allantoic entoderm, in which the initial multiplication of the virus occurs. A new multiplication of active particles follows the first virus generation; these particles use the still unaffected cells of the allantoic sac, etc.

Therefore, during a period of a 40-hour incubation period several virus generations may occur, the number of which is determined by the presence of sensitive tissue in the allantoic cavity.

Where there is a gradual inclusion of new areas of sensitive tissue a recovery from the initial pathology is possible. The regenerated epithelium may prove to be resistant to the virus multiplication, but, apparently, it is capable of assuring a nonpermeable condition of the sac for reabsorption and breakthrough of the virus beyond the limits of the allantoic cavity.

In work with certain influenza virus strains it has been observed that after a copious accumulation of active virus in the allantoic cavity an inactivation of it occurred, and the development of the embryo culminated in the hatching of a chick. This speaks for the fact that virus multiplication was of a local nature and that involvement of the sensitive entodermal cells within the allantoic sac was not associated with dissemination of the pathogen (Gorbunova, 1949).

The injection of the concentrated virus produces extensive pathology of the entodermal epithelium, the desquamation of large sections of tissue and the penetration not only of the virus but also of toxic metabolic products from the allantoic cavity into the embryonic organs. As a result of simultaneous pathology of the entire entoderm the function of the allantois is impaired. No sensitive cells

are found in the allantoic cavity in the new virus generation; the virus particles die out or gain access to the sensitive amniotic tissues and to the tissues of the yolk sac and chorioallantoic membrane through the injured entoderm, which involves a generalization of the infection and death of the embryo (Gorbunova, 1953).

When virus material is applied to the surface of the chorioallantoic membrane infection of the aliantoic fluid and of other parts of the embryo is possible.

The transfer of the infection from the surface of the membrane to the allantoic cavity is apparently accomplished either through the blood or lymphatic vessels or directly through injury of the mesodermal layer, lying between the ecto- and entoderm of the chorioallantoic membrane, by the virus.

After amniotic infection the virus spreads through the amniotic fluid, oral cavity, trachea and digestive tract, which can be shown readily by injecting dye along with the virus (trypan blue). Further multiplication of the virus occurs in the cells of the amnion and respiratory passages of the embryo, and the virus enters the allantoic cavity through the digestive tract with the circulating fluid, and there its multiplication can occur also (Burnet, 1940, 1941).

The distribution of the influenza virus in the chick embryo is readily demonstrated through the use of the methods of fluorescent antibodies. For this purpose the globulin fraction of the immune serum is combined with fluorescein propionate (Koons and Kaplan, 1950), and the tissues under study are treated with the serum which combines with the virus, if it is present in the tissues. Watson and Koons (1954) showed that with amniotic infection the virus accumulates in the amniotic epithelium, epithelium of the epidermis and respiratory tract of the embryo (Fig. 110, 111), also penetrating into the epithelium of the allantois and, less often, of the peritoneum. On cytological examination the stain appeared first in the nuclei and then in the cytoplasm (Fig. 112).

The condition which the virus encounters after being introduced into the yolk sac are different from the conditions in the allantoic cavity.

On the other hand, the virus and the contents of the yolk sac enter into the embryonic organism by means of absorption, that is, through the vascular system, regardless of the capacity of the given virus for multiplication in the cells of the yolk entoderm; on the other hand, in the event this tissue is sensitive to the given virus the infection may spread along the course of the entoderm from the yolk through the yolk stalk into the tissue of the

intestinal entoderm of the embryo.

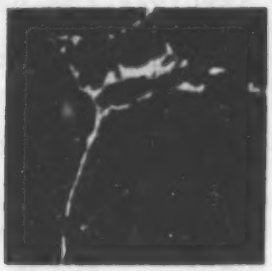

Fig. 110. Influenza Virus in Epithelium of the Larynx Shown by Means of Fluorescent Antibodies (Watson and Koons, 1954).

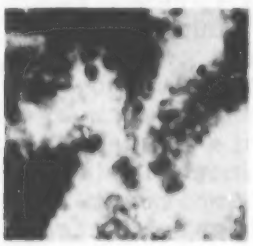

Fig. 111. Influenza Virus in Bronchial Epithelium. The Virus is in the Nuclei and Cytoplasm (Watson and Koons, 1954).

Fig. 112. Arrangement of the Virus in the Cell Cytoplasm. The Nuclei are Free of the Virus (Watson and Kaplan, 1954).

Therefore, after infection in the yolk sac cavity a continuous infection occurs of the embryo itself in case it is sensitive to the given virus. Therefore, cultivation in the yolk sac usually involves the death of the embryo (Gorbunova, 1953).

Multiplication of the influenza virus in the chick embryo can be divided into three stages: the lag-phase, where the virus does not accumulate in considerable quantities; the accumulation phase and the stage where the virus dies out.

In the lag-phase the virus is fixed by susceptible cells and reacts with the inhibitors of the egg. The mechanism of this reaction is apparently similar to the reaction of the influenza virus with the erythrocytes. Adsorption on the cells is associated with an enzymic destruction of the muco-protein substance (receptors) by means of the virus enzyme (hemagglutinin). If the cells are not viable, elution of the virus occurs after destruction of the receptors, as occurs when the virus reacts with erythrocytes. If the susceptible cells are viable, no elution occurs, and a further reaction of the virus with the cell takes place (Burnet, 1951).

Since there is a considerable quantity of inhibitor (ovomucin) in the chick embryo, during the first few hours of multiplication the presence of the virus is masked by the inhibitor. At this time, the virus can not as yet be demonstrated in the hemagglutination reaction, but can be demonstrated in the complement-fixation reaction. If the allantoic cavity is first treated with a receptor-destroying enzyme (filtrate of a cholera vibrio culture), a parallelism is observed in the increase of titers in the hemagglutination reaction and in the complement-fixation reaction in connection with the fact that the egg inhibitor has been eliminated (Liu and Henle, 1951). After considerable treatment of the allantoic cavity by the cholera vibrio enzyme a destruction occurs not only of the inhibitors but also of the receptors, in connection with which the virus is poorly adsorbed by the cells, and the influenza virus does not multiply in a considerable number of the embryos (Stone, 1947).

The penetration of the virus into the cell, however, apparently is not directly related to the destruction of mucoids by the virus enzyme. Fazekas de St. Groth (1948) injected active or heated virus (Lee) into the allantoic cavity, after which (immediately or after three hours) he introduced the receptor-destroying enzyme of the cholera vibrio into the allantoic cavity for the purpose of removing the virus remaining on the surface of the cells. After this procedure the content of free virus was determined in

the allantoic cavity. Under these conditions, no free virus could be found in the allantoic cavity. If the allantoic cavity was first treated with formalin the virus remained in the fluid. The author considers these data the result of penetration of the virus inside the cell after fixation of it on the cell surface. By analogy with the mechanism of adsorption and subsequent absorption of colloidal dyes (colloidopexis) he calls the mechanism of penetration of the virus into the cell "viropexis."

After the lag-phase a rapid increase occurs in the quantity of virus in the embryo. On serial investigations an increase in the infectious titer, hemagglutinating titer and complement-fixing titer of the antigen is noted. The maximum virus titers determined by these three methods are reached in the first two days for the A and A-prime and during the course of the first three days for B viruses.

During this time several multiplication cycles of the virus occur in the tissues of the embryo and it goes out into the embryonic fluid (Cairns, Edney and Fazekas de St. Groth, 1952; Cairns and Mason, 1953). The first cycle lasts five or six hours for the A viruses; eight to ten hours for the B viruses (Henle and Rosenberg, 1949).

According to Blumenthal and co-authors (1950), the multiplication of the influenza virus in the chick embryo and the exit of virus possessing infective activity into the allantoic cavity occurs over the course of several cycles, after each of which a latent period follows. In Fig. 113 four such cycles are shown in the course of 24 hours of incubation. Here, the hemagglutination titers increase later than the infective activity titers, and during the first 12 hours of growth and while it has a comparatively high degree of infective activity (10^{-5}) the allantoic fluid does not contain any hemagglutinins.

On subsequent days a rapid extinction and destruction of the virus occur. Gorbunova (1949) has shown that the hemagglutinin titer falls rapidly after four days of incubation and disappears on the seventh day.

The quantity of virus formed in the chick embryo which possesses hemagglutinating activity depends on a number of conditions.

Using massive infective doses, just as when minimum doses are used, the quantity of hemagglutinin formed is less than for average doses (Henle and Chambers, 1942; Henle and Henle, 1943, 1944). The optimum yield of hemagglutinating virus is observed when about 100 LD_{50} of the virus are injected into the allantois. The maximum accumulation of hemagglutinin for the A virus occurs between 36 to 48 hours of incubation at a temperature of 35-37°. For nine-ten-day embryos the temperature optimum is 37°;

for 10-11-day embryos, 37° (Miller, 1944).

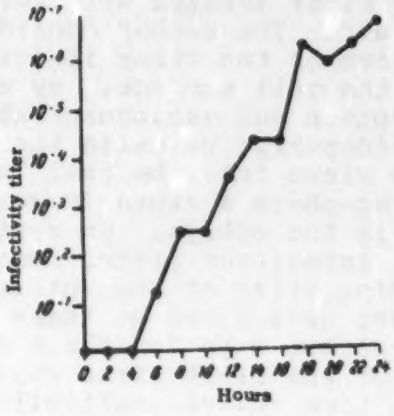

Fig. 113. Dynamics of Multiplication of Influenza A (PR8) Virus in the Chick Embryo During the Course of 24 Hours (Blumenthal and others, 1950).

The phenomena of autointerference may be observed in the group of influenza viruses in chick embryos.

The phenomena of autointerference were described by Henle and Chambers (1941) and Henle and Henle (1943, 1944). The essence of these phenomena consists in the fact that when large quantities of virus are injected in the embryo the virus accumulates in lesser titers than after infection with small doses of the virus.

Signs of interference may be observed between active and inactive viruses. A study of the mechanism of this phenomenon has made it possible to clarify certain rules and regulations of influenza virus multiplication in the tissues. Isaacs and Edney (1950, 1951) made a study of interference between heated (or formalinized) and active influenza A viruses. According to their data, heated or formalinized virus (MEL strain) injected an hour before infection with live active virus (PR8 strain) inhibits or completely checks the development of the latter. The minimal interference dose, that is, the quantity of killed virus which completely stops the development of 100 LD_{50} of active virus is equal to 300-1000 agglutinating doses for the heated and 2000-4000 doses for the formalinized virus. In subsequent experiments it was shown that the minimum interference dose is ten times less than the quantity of virus which can be adsorbed in the embryo and that the injection of a minimum interference dose into the

allantoic cavity does not prevent the adsorption of 100 LD_{50} of the live virus by the cells. Therefore, the phenomena of interference can not be explained by the blocking of receptors by the inactive virus. Interference occurs, apparently, intracellularly. The live virus penetrates into the virus-treated cells, disappearing from the surface of the chorio allantoic membrane, on the basis of which the authors conclude that interference occurs in the late stage of the multiplication cycle. A comparison of different strains shows that the higher the enzymic activity of the strain the higher its interference activity (for example, the variants of the WS virus create the inequality NWS > WSE > WSM). By using heating and formalin treatment of the viruses the authors showed that thereby the interference strength of the strains is markedly reduced or completely lost, which is associated with a loss of affinity for the inhibitor of the chorioallantoic membrane (this inhibitor is different from the inhibitor of the egg white and that of the allantoic fluid). Since this is an intracellular inhibitor, the authors draw the conclusion that interference between killed and active virus occurs within the cell and the nature of it consists of the binding of this inhibitor, necessary for virus multiplication, by the killed virus. The phenomena of autointerference can occur through the use of viruses inactivated by different methods: heating, prolonged standing (Henle and Henle, 1944), by ultraviolet irradiation (Ziegler and others, 1944).

A study of the autointerference phenomena made it possible to understand certain distinctive features of influenza virus multiplication.

It was pointed out above that after infection of embryos with large doses of the virus (undiluted allantoic fluid) lower infectious virus titers are obtained than after infection with small doses of the virus (dilute allantoic fluid). Magnus and Gard (1947) showed that the quantity of hemagglutinin in both cases is large, but in the former case the infectious activity of the virus is markedly reduced. Therefore, in both cases the same quantity of virus is formed in the embryo, and the viruses are identical in their antigenic structures but have different infectious activities.

Magnus (1951-1952) believes that the injection of concentrated PR8 virus into the allantois leads to the production of "incomplete" virus particles which are identical in their antigenic structure and enzymic activity to the original virus but do not possess infectious activity. The author believes that "the immaturity" of the virus occurs as the result of the overloading of susceptible cells by virus particles. The incomplete virus, reacting with the

cell, interacts with its receptors but is incapable of multiplying. After injuring the cell receptors it makes the penetration and development of the "mature," complete virus in it impossible. The essence of the so-called "autointerference phenomena," where the injection of massive doses of virus reduces the production of it in the chick embryo and in the body of susceptible animals, consists of this.

Fazekas de St. Groth and Graham (1954) studied this phenomenon in ten influenza virus strains (A, A-prime, B and Swine) and showed that various strains possess this property in varying degrees: the property of producing "incomplete" virus is greatest in the PR8 strain; least, in the Lee strain.

McKee (1951) showed that this "incomplete" virus loses the toxic properties existent in the original virus.

In a number of his works Hoyle (1948, 1950) attempts to apply the hypothesis of bacteriophage reproduction propounded in recent years for the purpose of explaining the mechanism of influenza virus multiplication. He believes that the virus particle, penetrating into the cell, disintegrates into smaller particles which correspond to the soluble antigen. In this form the virus multiplies in the cell, and on leaving it larger aggregates are formed which correspond to the elementary bodies.

As confirmation of his data the author presents works on the incomplete virus, which, in his opinion, is identical with the aggregates of soluble antigen without hemagglutinin. In this work data are presented concerning the quantitative determination of the soluble antigen and a drawing of the formed virus particles is shown after they have left the cells as observed through microscopy of virus cultures in a dark field; these are strikingly similar to the morphological findings of Draganov described in the second chapter.

In the following work, the author (Hoyle, 1952) obtained two fractions -- hemagglutinin and soluble antigen -- by means of treatment of the virus with ether and adsorption of it onto erythrocytes, and he studied their properties. The soluble antigen proved to be a ribonucleoprotein having dimensions of 12-25 millimeters and possessing complement-fixing activity; the hemagglutinin is an enzyme of protein nature.

In the last of his published works, Hoyle (1954) comes to the conclusion, on the basis of morphological pictures obtained in the examination of sections of chorioallantoic membrane infected with influenza virus, that the infectious particle represents a fragment of cell cytoplasm and consists of compact masses of virus protein enclosed within the cell membrane.

The data presented have been obtained in experiments with the injection of virus into the allantoic cavity. If the virus is applied to the outer surface of the chorion, the hemagglutinin and soluble antigen do not accumulate, and the infectious activity is increased slightly (by 100 times). If larger quantities of the virus (10^8 LD_{50}) are injected the quantity of soluble antigen is proportional to the quantity of virus injected; hemagglutinin can be found only in the form of traces if the embryo is treated with the cholera vibrio enzyme (Isaacs and Fulton, 1953).

Attempts at constructing a quantitative theory of influenza virus multiplication in the chick embryo are represented in the work of Cairns, Edney and Fazekas de St. Groth (1952). The authors start with the principle that virus multiplication in the cells occurs in the form of several cycles each of which includes fixation of the virus by susceptible cells, multiplication of the virus in the cell, and exit of the virus particles from the cell. On the basis of mathematical calculations, reinforced by experiments, the authors found that in the first multiplication cycle about $10^{8.2}$ allantoic cells produce a virus yield wherein there are 61-73 virus particles per cell.

In the three multiplication cycles studied, the liberation of the complete and incomplete virus occurred simultaneously. The first cycle lasted 7 1/2 to 9 hours; the second, 5 to 7 hours; the third and fourth, 4 1/2 to 5 1/2 hours. One hemagglutinating dose contained $10^{6.26}$ LD_{50} of the virus.

Electron microscopic study of the process of virus multiplication has led certain authors to the conclusion that virus particles are not formed within the cells but rather in the peripheral portions of the cytoplasm (Eddy and Wyckoff, 1950). Thereby, no division of the virus particles in equal halves occurs, as is observed in bacterial multiplication, but rather a kind of growth of the virus particles out of the cytoplasm (Figs. 114-116) in the form of spherical rod-like structures, chains and filaments (Wyckoff, 1953). Similar data have been obtained by Murphy and Bang (1952), the microphotographs from whose work are reproduced in Figs. 117-119. The authors also come to the conclusion that the influenza virus does not penetrate into the cells but rather develops on the surface of them in the intercellular spaces, reproducing from peripheral portions of the cytoplasm. The authors note that the filamentous structures are the end stage in the development of the influenza virus, in the disintegration of which the spherical elementary bodies are formed.

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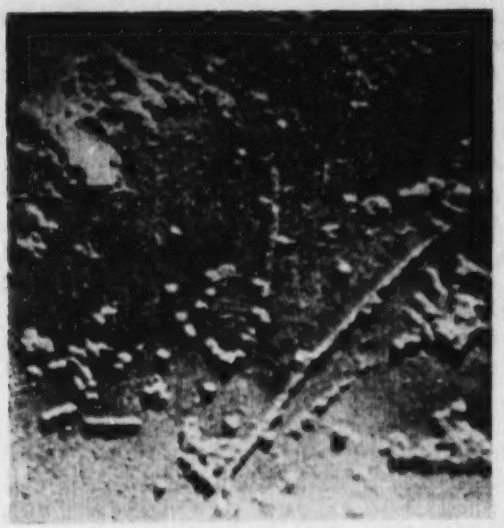

Fig. 114. Purified Influenza Virus Suspension. Rods and Spheres of Constant Diameter. Magnification 16,000 (Wyckoff, 1953).

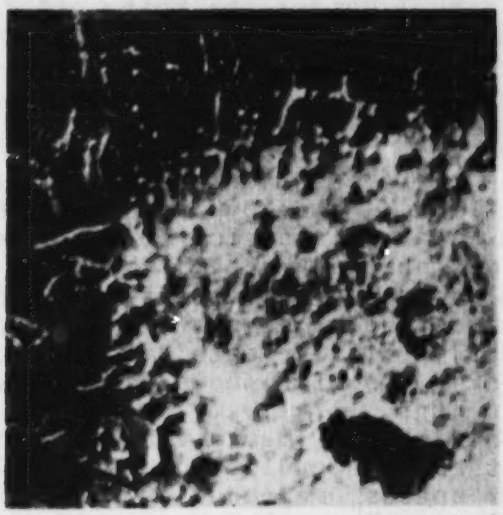

Fig. 115. Section of Chorioallantoic Membrane Infected With Influenza Virus. Rods and Spheres are Grouped at the Margin of the Cell Cytoplasm. Magnification 12,000 (Wyckoff, 1953).

Fig. 116. Rods Representing Prolongations, As It Were, of the Cytoplasm. Magnification 20,000 (Wyckoff, 1953).

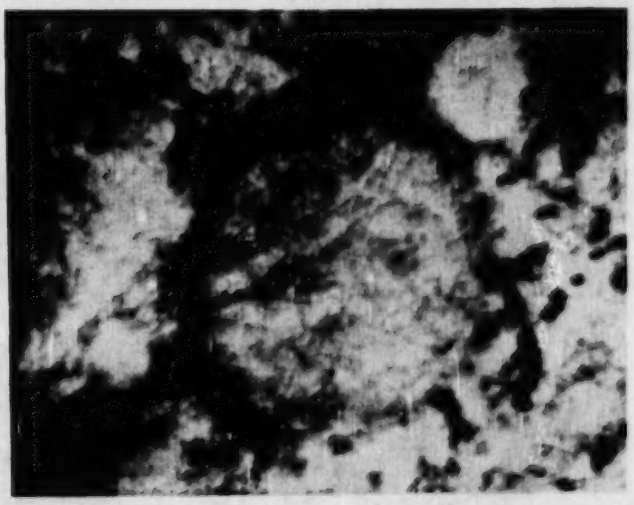

Fig. 117. Influenza A (PR8) Virus on the Chorioallantoic Membrane on a Tissue Culture According to the Maximow Method. Filaments and Spheres Are Seen on the Surface of the Nucleus and Cytoplasm. Magnification 6,200 (Murphy and Bang, 1952).

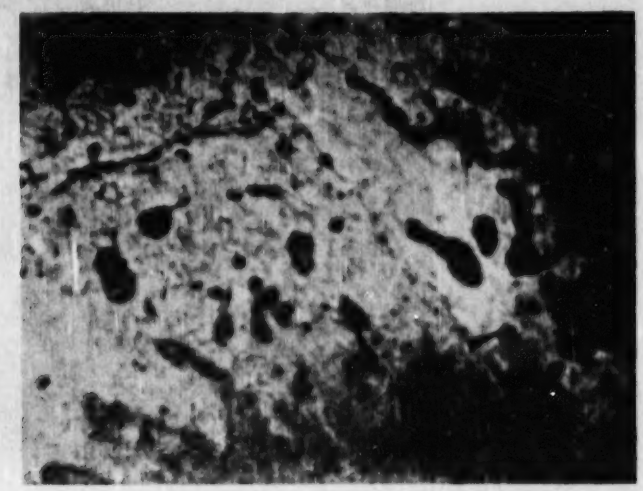

Fig. 118. Culture of Swine Influenza Virus (V15). Transection of Three Cells. The Virus Is Found Only in the Left Lower Cell (Murphy and Bang, 1952).

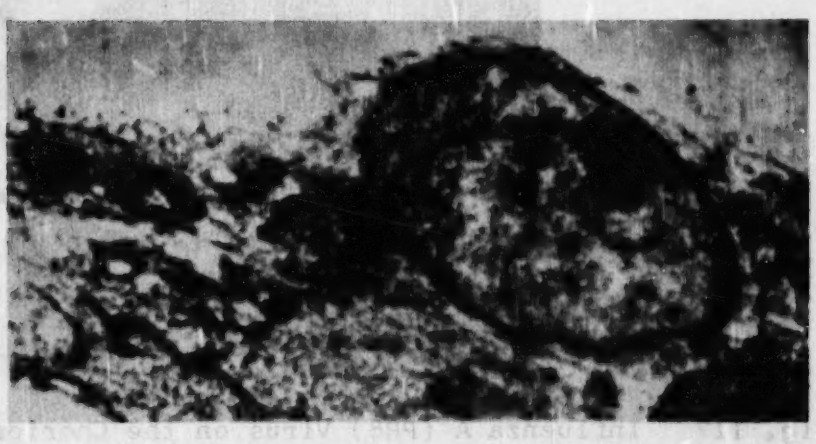

Fig. 119. Section of Chorioallantoic Membrane of Chick Embryo Infected With Swine Influenza Virus After 48 Hours of Incubation. Short Filaments With Terminal Nodules and Spheres Along the Borders of the Cells (Murphy and Bang, 1952).

The analysis of these morphological pictures should be approached with great caution, however, because we know little about the behavior of the virus in the cell cytoplasm. It is entirely possible that the methods of electron microscopy used do not permit the detection of the virus in the cytoplasm in those cases where its density is no different from the density of the cell cytoplasm, and that it is in a state in which it may be detected with the electron microscope only after it comes out of the cell.

Multiplication of influenza viruses in chick embryos does not produce any gross morphological changes. This, however, does not apply to all strains. In the previous chapter it was mentioned that strains of influenza virus adapted to chick embryos cause their death (Solov'yev and Gutman, 1953). The pathology of embryos produced by virus pathogenic for them has been described in greater detail by Burnet and co-authors (1936), by Westwood and certain other investigators (Burnet, Lush, 1938; Westwood, 1952, 1953).

Burnet (1936) studied the pathology produced by influenza virus adapted to chick embryos (1936). Macroscopically, this pathology looks like scattered foci of thickening of the chorioallantoic membrane (Fig. 120). On histological examination these thickenings represent foci of necrosis and inflammatory infiltration (Fig. 121). After prolonged passage of the virus the latter causes hemorrhages into the brain and muscles.

Fig. 120. Foci of Thickening of the Chorioallantoic Membrane of Chick Embryo Infected With Influenza Virus (Burnet, 1936).

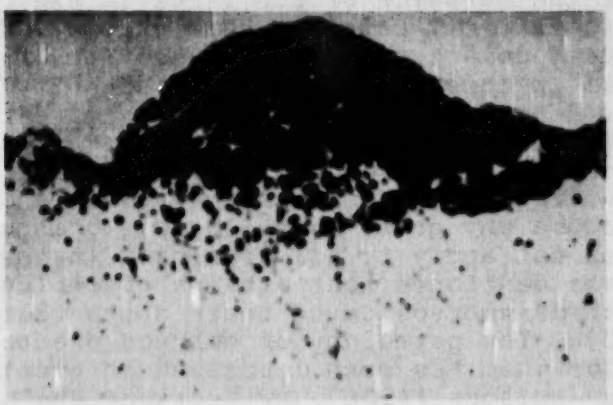

Fig. 121. Histologic Section of One of the Foci of Thickening Pictured in Fig. 120 (Burnet, 1936).

Westwood (1952) described the pathological changes produced in chick embryos by A and B viruses. Embryos infected at the age of 10 days lag in their development, a hyperemia of the cutaneous blood vessels is noted, with small hemorrhages into the skin and muscles, edema of the tissues, hyperemia of the internal organs and meninges.

On microscopic examination edema and hyperemia of the skin, muscles, and internal organs, and degenerative changes in the liver (Fig. 122), kidneys (Fig. 123), lungs (Fig. 124), meninges (Fig. 125) are found. However, these changes are nonspecific and are observed in other virus infections as well as after the effect of bacterial toxins. At the age of 11-12 days, the sensitivity of embryos to the influenza virus falls off markedly.

Ritova (1953) observed hemorrhagic signs in chick embryos produced by A-prime virus (Krav). The general character of the pathology is shown in Fig. 126. On histological examination large hemorrhagic foci are noted in the lungs and muscles, necroses and autolysis of muscle tissue.

Therefore, the nature of the pathology in chick embryos and the histopathological changes observed depend in large part on the biological characteristics of the influenza virus strains studied.

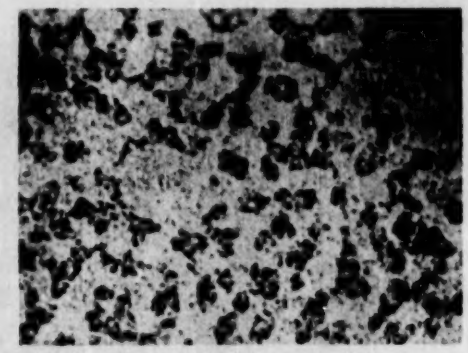

Fig. 122. Liver of Chick Embryo Infected With Influenza Virus (Westwood, 1952).

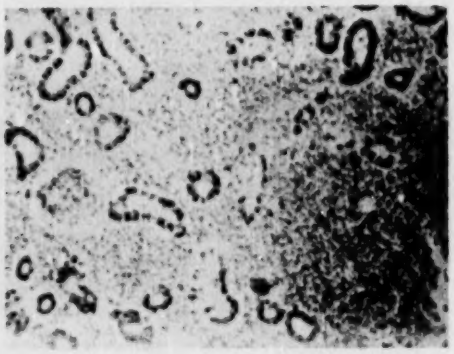

Fig. 123. Kidney of Chick Embryo Infected With Influenza Virus (Westwood, 1952).

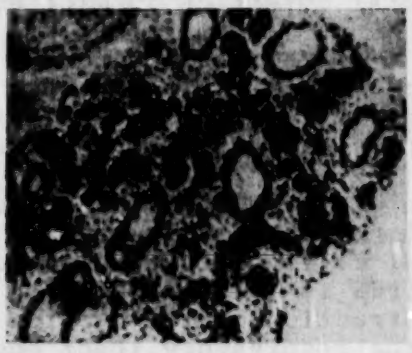

Fig. 124. Section of Lung of Chick Embryo Infected With Influenza Virus (Westwood, 1952).

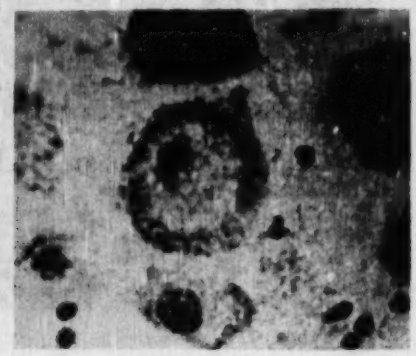

Fig. 125. Section of Spinal Ganglia of Chick Embryo Infected With Influenza Virus (Westwood, 1952).

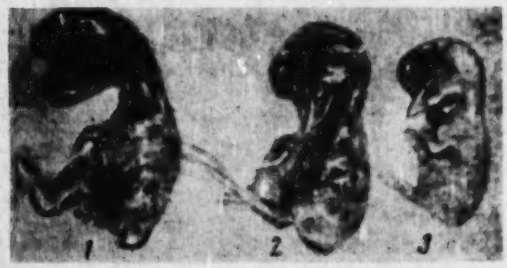

Fig. 126. Hemorrhagic Pathology in Chick Embryos Infected With Influenza Virus.

1, 2 -- hemorrhages; 3 -- normal embryo (Ritova, 1953).

40. Experimental Infection in Mice and Other Laboratory Animals

Multiplication of the virus in the bodies of mice shows a number of features in common with its multiplication in chick embryos in the initial stages of the infection.

In the initial stage of the infection after intranasal introduction of the mouse-adapted virus, it is fixed in the epithelial cells of the lungs and bronchi. In this lag-phase the virus is not found in the free state. Simultaneously, a destruction of the mucoids (receptors and inhibitors) of the respiratory tract occurs. In this stage of the tissue a complement-fixing antigen can be found, but the hemagglutinin appears later, after the virus multiplying

in the cells enters the respiratory passages with the secretion in the form of elementary bodies. Complete exhaustion of the susceptible cells does not occur, because processes of repair and immunity develop; antibodies may be found beginning with the third day (Fazekas de St. Groth, 1950).

The introduction of the receptor-destroying cholera vibrio enzyme into the lungs makes mice partially resistant to subsequent infection with influenza viruses (Stone, 1948). The dynamics of virus multiplication are represented in the following form in their general outlines. After infection the quantity of virus does not increase in the lungs for the first five or six hours. Then a period follows when there is a rapid increase in the quantity of virus in the lungs which reaches its maximum titer after 24-48 hours. If the mice do not die at this time the virus titer remains unchanged up to the sixth day, after which it decreases with the production of immunity (in connection with the production of antibodies). After the introduction of sublethal doses of the virus its maximum titers are usually 100 times lower than after infection with lethal doses. Increase in the number of lethal doses does not lead to any further increase in the maximum virus titers in the lungs (Taylor, 1941).

In the use of the complement-fixation reaction traces of the influenza virus antigen in mouse lungs can be found as early as after four hours, and after 24-48 hours its concentration reaches maximum titers, beginning to fall off slightly after 72 hours (Peterson, 1943).

Gaydamovich and Marennikova (1952) studied the multiplication of virus in mouse lungs after infection of them with an unadapted strain which caused an asymptomatic infection. The most sensitive method of detecting the virus was the infection of chick embryos; the complement-fixation reaction was next in sensitivity, and least sensitive was the hemagglutination reaction. Therefore, the process of virus multiplication in an asymptomatic infection is similar in its general outlines to the multiplication of the pathogenic virus.

The distribution of the virus in the bodies of mice and other susceptible animals has been studied in detail by Smorodintsev, Ostrovskaya and Drobyshevskaya (1938). In contradistinction to Smith, Andrewes and Laidlaw (1933), who believe that the causal agent of influenza is a purely pneumotropic virus, the authors established the existence of an extensive dissemination of the virus in the bodies of animals after intranasal infection. After the inoculation of 1000 MLD the virus was found on the fourth day in the lungs, pleural cavity, blood, spleen, liver, bile,

urine, and was absent from the small intestine. the quantity of virus in the lungs increases rapidly, and by 24-48 hours after infection it reaches 10^6-10^7 MLD, after which a slow decrease in it occurs. In mice which die the virus titer in the lungs is considerably less than in animals killed 24-48 hours after the infection. Similar data have been obtained by Fal'kovich (1944).

The virus, after being injected intravenously in large quantities (50,000 MLD) or intraperitoneally, dies rapidly in the body. In 72 hours it can be found in the lungs in the absence of any pathological changes (Smorodintsev and others, 1938; Panthier and others, 1948). A very similar picture of virus distribution in the body is found after experimental influenzal infection of ferrets and young pigs.

The reaction of the influenza virus with the susceptible organism is, as a matter of fact, more complicated than it appears from the experiments presented on the study on the multiplication cycle of viruses. A series of special experiments performed by Kravchenko and Sekretta (1950), constitute evidence of this. Based on previous works concerning the cumulative effect of bacterial toxins, the authors performed experiments on the study of the effect of repeated infection of mice with sublethal doses of influenza virus on the development of the infection.

In these experiments it was shown that the repeated daily injection of the influenza virus into mice through the nose under ether anesthesia in quantities 10-100 times less than a single lethal dose caused the development of a typical infectious process which led to the death of the animals sooner than after the injection of a single lethal dose.

In evaluating the data of these experiments, the authors come to the conclusion that repeated contact of the influenza virus with susceptible cells involves a change in the body's reaction to the influenza virus similar to what occurs after the effect of bacterial exotoxins.

In the next series of experiments the authors studied the significance of intervals and number of times mice were infected with sublethal doses of the virus for the development of the infection. They showed that one or two injections of the virus are inadequate for producing death of the mice, and death occurs after the virus is injected three to six times. The time of death of the mice is reduced or lengthened depending on the magnitude of the intervals between the separate injections of small doses of serum. In connection with the obtaining of neurotropic strains of influenza virus experiments were performed on the study of influenzal infection in mice caused by the intracerebral injection of the virus.

After the intracerebral injection of the neurotropic virus (MWS strain) with preliminary destruction of the receptors by means of the injection of filtrates of the cholera vibrio into the brain a parallel increase in infectious activity, hemagglutinin and complement-fixing antigen occurs, and these reach their maximum titers on the third day after infection. The ratio of infectious and hemagglutinating titers (I/HA) reaches 10^6. If the neurotropic strain (WS, PR8, Lee) is injected, these three indices reach their maximum after nine to eleven hours, whereby the ratio I/HA does not exceed $10^{1.75}$. Therefore, in this case only a single multiplication cycle occurs with the formation of an incomplete virus (Schlesinger, 1950). Up to 15 percent of the virus injected into the brain passes rapidly into the blood stream (Cairns, 1941). Preliminary treatment of the brain with cholera-vibrio filtrates which destroy the receptor mucoids increases the resistance of the mice to the neurotropic influenza virus by 100 times. Thereby, the concentration of the Francis inhibitor in the brain drops sharply (by 99.6 percent), being regained only on the 20th day (Cairns, 1951). The same effect is observed after the preliminary injection of sublethal doses of cholera-vibrio filtrates of virus strains which are not neurotropic (Wagner, 1952).

Histopathology of Experimental Influenza. In ferrets, following intranasal infection with influenza virus, characteristic pathology of the respiratory tract develops (Francis and Stuart-Harris, 1938). As early as the end of the incubation period (a day after infection) a small amount of exudate appears in the lumina of the bronchi (Fig. 127, a, b). On the second day of the disease necrosis and desquamation of the cylindrical epithelium of the bronchi, edema and infiltration of the submucosa and the formation of an exudate in the lumina of the bronchi develop (Fig. 127, c).

In the next three or four days a complete desquamation of the ciliated epithelium occurs throughout the respiratory tract (Fig. 127, d), the submucosa is abundantly infiltrated with polynuclear cells, lymphocytes, plasma cells and monocytes. Beginning with the fifth or sixth day reparative processes (Fig. 127, e) develop: at first, instead of the cylindrical [columnar] epithelium a transitional monolayered epithelium develops. In the interstitial tissue fibroblasts appear in place of the mononuclear cells and the polynuclear cells. The upper layer of bronchial epithelium sloughs off (Fig. 127, f); the underlying cells are converted into ciliated columnar (Fig. 127, g) epithelium, and the lumina of the bronchi are cleaned of residues of the exudate (Fig. 127, h). The entire process of recovery of the normal mucosa is completed three or four weeks

after the infection.

Fig. 127. a, b, c, d, e, f, g, h. Histopathology of Experimental Influenza in Ferrets. Explanation in text (Francis and Stuart-Harris, 1938).

Brightman (1936) found similar pathology in ferrets beginning with the second or third passage. Microscopically, they had the appearance of dark-red foci. On histo-

logical examination a peribronchitis was found with round-cell infiltration, inconstant hyperemia, partial desquamation of the bronchial epithelium, and exudate in the lumina of the bronchi.

The histopathology of experimental influenza in white mice has been studied in detail by Dal' (1938). Four hours after intranasal or inhalation infection circulatory disorders were noted in the animals' lungs with the initial signs of bronchiolitis: congestion of the blood vessels, swelling of the cytoplasm of the columnar epithelial cells, and an accumulation of lymphoid cells in the peribronchial tissue (Fig. 128, a).

During the next few days the pulmonary edema and bronchiolitis progress. Masses of mucus with desquamated epithelial cells, degenerating leukocytes and lymphoid cells (Fig. 128, b) appear in the bronchial lumina. After two or three days signs of pneumonia develop, at first in the form of small pneumonic foci with the predominance of leukocytes, lymphoid cells, alveolar macrophages and solitary leukocytes in the exudate. The pneumonic foci develop peribronchially. After four or six days, the epithelial lining of the bronchi is completely destroyed, intense circulatory disorders develop with extensive foci of hemorrhage, catarrhal or small-focus pneumonia with atelectases, pulmonary edema and diapedesis of the erythrocytes into the lumina of the alveoli (Fig. 128, c, d). In this stage of the disease the death of the animals occurs, whereby the rapidity of development of the process depends on the dose and the virulence of the inoculated virus for the mice. In the other organs hyperplasia is noted in the spleen, and sometimes degenerative processes are noted in the liver and kidneys.

With association of the influenzal infection with the hemolytic streptococcus, pneumococcus or Bacillus influenzae the pulmonary pathology is more pronounced and has the nature of catarrhal or purulent-necrotic pneumonias (Fig. 128, e); the pathology in the spleen, liver and kidneys if found less often.

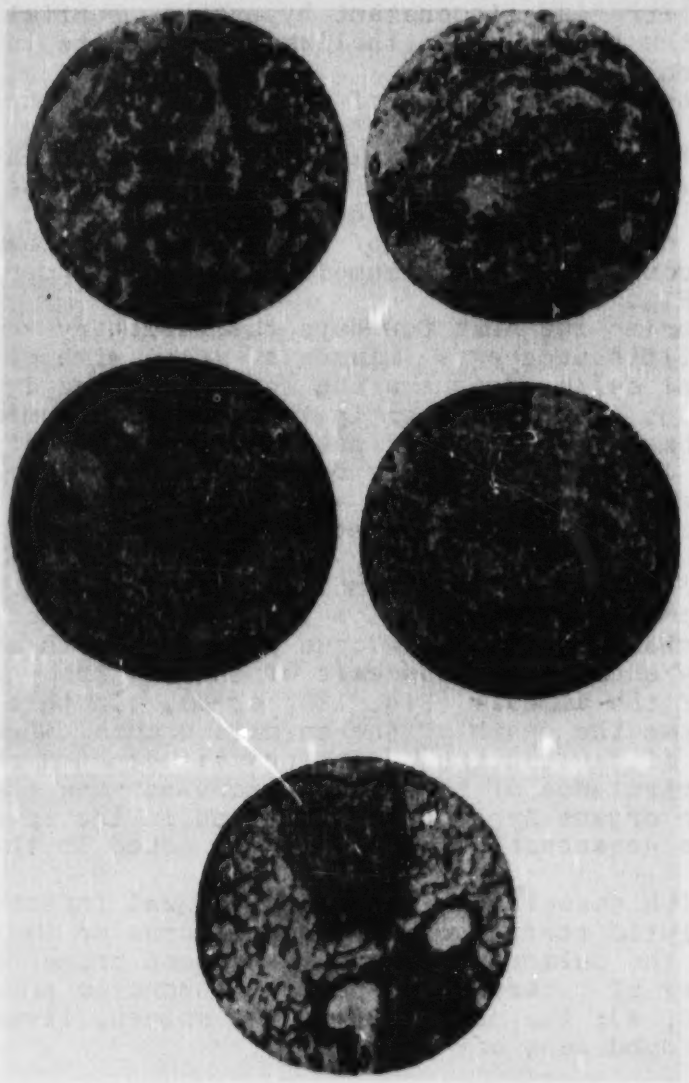

Fig. 128. a, b, c, d, e. Histopathology of Experimental Influenza in Mice. Explanation in text (Dal', 1938).

Fig. 129. a, b, c. Types of Pulmonary Pathology in Experimental Influenzal Infection in Mice (Zil'ber, Fal'kovich and Arkhina, 1937).

Zil'ber, Fal'kovich and Arkhina (1937), on histological examination of mice infected with the influenza virus ($A\text{-}M_{13}$ strain), found three types of pathology: a catarrhal-hemorrhagic bronchopneumonia, bronchopneumonia with peribronchitis and necrotic bronchopneumonia. In the first case, hyperemia was noted in the lungs with the accumulation of leukocytes and serous fluid containing erythrocytes, and serous-hemorrhagic permeation of the periphery of the affected areas in the bronchi and alveoli (Fig. 129, a). In the second case, the involvement of the bronchi was insignificant; the peribronchial and perivascular spaces were widened, and a seropurulent exudate accumulated in them; the pulmonary interstitial was inflamed and edematous (Fig. 129, b); the interalveolar areas were infiltrated with leukocytes, and not uncommonly a sero-purulent pleurisy develops. This type of pathology is most often observed. The third type of pathology is characterized by a partial or complete necrosis of the bronchial wall and of the corresponding section of the lung; along the periphery of the necrotic focus a hemorrhagic impregnation of the lung is observed (Fig. 129, c)

Similar data have been obtained through a pathological study of experimental influenzal infection in mice by Lauer and Lozova (1939). According to their data, three types of pathology are encountered in the lungs of mice infected with influenza: 1) a circulatory disorder, edema of the lung, and slight inflammatory signs; 2) circulatory disorder, infiltrative inflammatory process around the bronchi, bronchioles, vessels and alveolar septa; 3) a bronchopneumonic process. They studied the changes observed after infection of rats, rabbits and susliks with the influenza virus.

According to Straub (1937), in mice infected with influenza virus a necrosis of the epithelium of bronchi and bronchioles with impregnation with fibrinous exudate, desquamation of the epithelium occurs, and sections of collapsed and dilated alveoli appear, and the lung tissue is edematous and hyperemic. With recovery a proliferation of the epithelium develops. The entire process in mice includes only the lower segments of the respiratory tract, in contrast to ferrets; the nasopharyngeal mucosa remains normal.

According to the data of Nelson and Oliphant (1949), when mice are infected with virulent strains of influenza virus edema begins to develop after three or four hours, and then there is infiltration of the interalveolar septa; after 18-24 hours, the alveoli are filled with a fibrinous exudate containing erythrocytes, lymphocytes and polynuclear cells. The bronchial epithelium becomes necrotic, and the bronchi are filled with casts and inflammatory cells.

In the animals which survive chronic processes develop with metaplasia of the alveolar epithelium and with damage to the bronchial lining.

Botsman (1952-1953) made a study of the histopathological changes in the argentophilic fibrillar structures of the basic intercellular substance in experimental influenzal infection. These structures contain an abundance of protein-polysaccharide substances which constitute the substrate of the enzymic effect of the influenza virus. The argentophilic fibers of the internal organs do not show any definite changes. In the lungs, at the early stages of the infection the formation of fibers occurred from structureless substance, which later became thickened and fragmented. Less pronounced changes were observed in the central nervous system.

Harford and Hamlin (1952) did not establish the existence of any damage to the ciliated epithelium of mouse bronchi during checking investigations when the mice were infected with lethal doses of the influenza viruses (PR8, WS, swine strains).

Kitayama, Sunakawa, and Fukumi (1953) studied the histological changes produced by the viruses of influenza and of Newcastle disease. They have come to the conclusion that the neurotropism of certain strains is associated with their capacity for multiplication in the ependymal cells, which are biologically similar to the bronchial epithelial cells.

In connection with this work it is fitting to compare the observations made by Sheftel' (1940) on anatomical changes in the central nervous system in influenza. After the infection of white mice with the influenza virus, Sheftel' was able to find pathological changes in the cerebral tissue as early as the second day after the infection, which increased gradually as time progressed. The changes amounted to a dilatation and inflammatory infiltration of the blood vessels, proliferation of the glial and histiocytic elements and alteration of the ganglion cells, that is, to encephalitis. These changes are observed chiefly in the anterior segments of the brain. A similar picture was observed by the author in the brains of ferrets after they were infected with the influenza virus.

Tret'yakova (1952) found changes in the olfactory receptors in mice infected with the influenza virus, necrosis of the olfactory cells, edema of the perineural sheaths of the olfactory nerve, and changes in the cornu Ammonis.

The histopathological picture of the trachea, bronchi and lungs after infection of mice with the influenza type B virus was the same as after infection with the type

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A virus (Oliphant and Perrin, 1942).

In one of the works (Pigarevskiy, 1953) an attempt has been made to understand the morphological changes in experimental influenzal infection not as a passive destructive process produced by the virus but rather as a reactive process. The author points out that the epithelial changes in the trachea and bronchi of mice in virus influenza have proved to be much more complex than what was found by other investigators. It is not a simple passive destruction of the epithelial cell which occurs but rather an active response of it, owing to which the virus is walled off and afterwards excreted from the cell. Thereby, it becomes accessible to the effect of other protective forces of the body and is also removed from the respiratory tract along with the degenerating cells. The epithelial cells which have been freed of the virus do not die as a rule. They rapidly recover and serve as a source of further regenerative proliferation of the epithelial layer.

In this process two groups of protective reactive processes are observed:

a) an intensification of the physiological secretion processes, which prevents the penetration of the virus into the epithelial cells;

b) after penetration of the virus into the epithelial cells reactive phenomena occur in them directed at walling off the virus (by means of formation of eosinophilic inclusion bodies) and the subsequent elimination of it from the cell (by means of the elimination of these inclusion bodies from the cell into the lumina of the respiratory tract). After this, a regeneration of the epithelium occurs which leads to a complete recovery of the integrity of the epithelial layer.

The author draws the conclusion that these reactions are evidently indisruptibly connected with humoral and other general protective factors in the organism.

41. Toxic Effect of Influenza Viruses

Influenza A, A-prime and B viruses possess a toxic effect. This effect was found after the introduction of massive quantities of the virus into the brain, abdominal cavity and intravenously in experiments on mice, rats, guinea pigs, rabbits and hamsters as well as after the introduction of the virus into the anterior chamber of the eye of rabbits and guinea pigs (Rickard and Francis, 1938; Evans and Rickard, 1945; Hale and McKee, 1945; Henle and Henle, 1945, 1946, 1948; Gol'bert and Peterson, 1948; Kempf and Harkness, 1950; Zakstel'skaya, 1950, 1951). Exhaustive investigations on the problem of toxicity of the

influenza virus with the application of new methods (Tarusov and Khayt, 1944) have been made by Zakstel'skaya (1953).

According to the data of Zakstel'skaya, the intracutaneous injection of allantoic cultures of the virus into mice, rabbits and guinea pigs is not associated with any kind of definite pathological reaction. The intravenous injection of allantoic cultures into rabbits and guinea pigs does not always give a constant effect, although not uncommonly death of the animals or the development of inertia and tremor in them is observed; at autopsy, hemorrhages are found in the liver and sometimes in the walls of the intestine. Similar data have also been observed by Henle and Henle (1946, 1948) in rabbits, guinea pigs and rats; by Pille (1950), in rabbits.

A distinct toxic effect is observed after the intravenous injection of the virus into mice. Eighteen to 24 hours after the injection of the virus the animals become inactive, tremor of the head develops and there are periodic convulstions; the animals die with increasing inertia or during convulsions, usually during the first two or three days after the infection. The pathological changes in the mice which died were the following: the lungs were anemic; the liver and intestinal walls were covered with a network of dilated capillaries; individual intestinal loops were distended and overfilled with a bloody content; in the pericardium and under the liver capsule and in the loops of small intestine there were abundant hemorrhages, and sometimes a bloody exudate in the pleural cavity (Fig. 130). On histological examination the lungs of the mice which died from the intoxication were not much different from normal lungs. In other internal organs disturbances were observed in the capillary circulation with foci of hemorrhages and necroses. The most marked changes were noted in the liver: foci of hemorrhages and necrosis of the liver lobules as a result of compression of the cells by the excess of blood and the impairment of their nutrition (Fig. 131). Similar changes occurred in the walls of the intestine (Fig. 132) and in the myocardium (Fig. 133).

After intracerebral injection of the virus into mice a tremor of the head and convulsions develop in the animals on the same day; the main part of the animals dies during the first three days. At autopsy a characteristic dull white color of the liver is found; hemorrhages in the intestine and the internal organs are less pronounced than after intravenous injection of the virus. The brain is not much changed (Zakstel'skaya, 1953).

Various strains of the influenza virus behave differently, although in general they all possess a more or less pronounced toxic effect.

Fig. 130. Mouse Which Died from Experimental Influenzal Intoxication.

1 -- hemorrhages in the internal organs; 2 -- loops of intestine filled with bloody content (Zakstel'skaya, 1953).

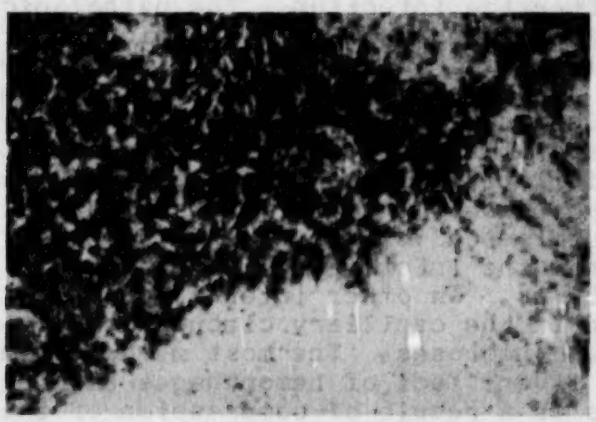

Fig. 131. Liver of Mouse Which Died from Toxic Effect of Influenza Virus; Marginal Necrosis of Lobule (Zakstel'skaya, 1953).

The virus, which produces a toxic effect after intracerebral injection into mice can not be passaged by this means, and it is possible to find residuals of the virus in the brain only during the first few hours (Henle and Henle, 1946).

The toxic effect of the influenza virus in mice after intraperitoneal injection of large doses of the virus has also been established in the experiments of Gol'bert and Peterson (1942). At the same time, an inflammatory process

also developed in the lungs which was less intense than after intranasal introduction of the virus.

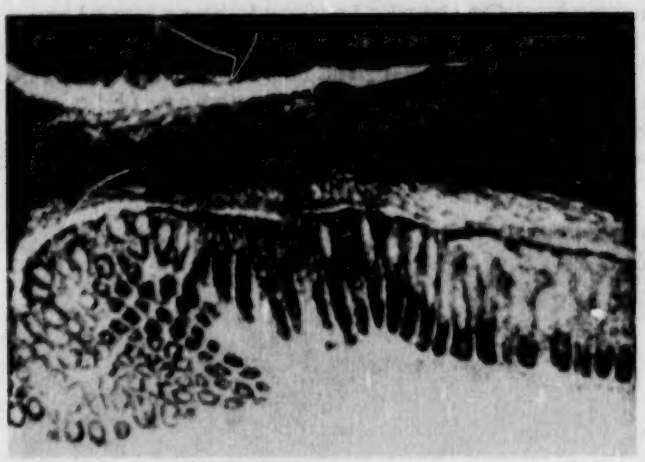

Fig. 132. Intestine of Mouse Which Died from Toxic Effect of Influenza Virus (Zakstel'skaya, 1953).

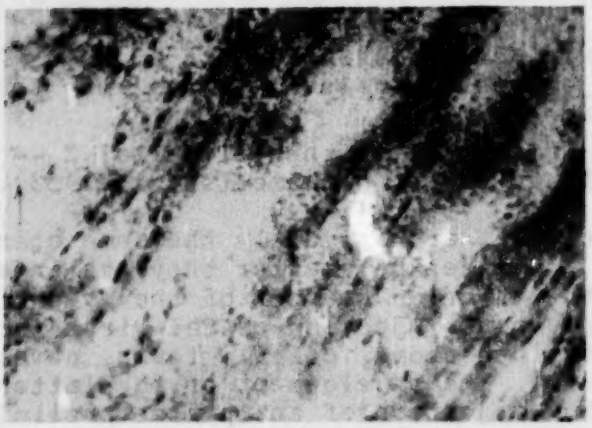

Fig. 133. Heart of Mouse Which Died from Toxic Effect of Influenza Virus (Zakstel'skaya, 1953). The arrow indicates hemorrhages in the muscle layer.

After the injection of the virus into the anterior chamber of the eye of rabbits and guinea pigs an injection of the scleral vessels is observed after 20-24 hours near the limbus, with hyperemia and swelling of the iris, and a

dilatation of the pupil. After two days, a keratitis develops; opacification of the cornea is most pronounced on the third-fourth day, after which a resorption of the edema begins and after 8 to 15 days the cornea has returned completely to normal. On histological examination of the cornea after a day vacuolization of the epithelial cells is observed; after two days, a necrosis of the epithelium and edema of the tissue of the cornea proper (Fig. 134). On subsequent days, the pathological phenomena undergo regression, and the corneal epithelium comes back to normal.

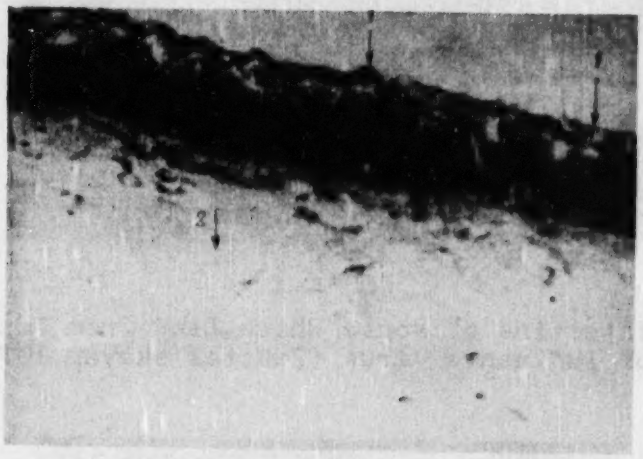

Fig. 134. Toxic Keratitis in Rabbits 48 Hours After the Injection of Influenza Virus Into the Anterior Chamber.

1 -- necrosis of corneal epithelial cells; 2 -- edema of the corneal tissue proper (Zakstel'skaya, 1953).

A reaction of approximately the same character is observed also in guinea pigs after they are injected with the virus in the anterior chamber of the eye.

On intravenous injection of the virus into rabbits the effect described above, chiefly fever, develops after 1 1/2 to 3 hours. The development of the latter may be prevented by the injection of antipyrine preliminarily (Wagner and others, 1949).

The comparative data concerning the pathogenic (toxic) effect of certain virus strains studied by Zakstel'skaya (1953), are presented in Table 55.

Table 55

The Effect of the Influenza Virus on Various Animals After Intravenous Injection

Material under investigation					Death of the animals after intravenous injection of the virus		
Tissue substrate	Type of virus	Strain of virus	Virus titer according to hemagglutination reaction	Dilution	Mice	Rabbits	Guinea pigs
Allantoic cultures	A	PR8	1:1 040	Undiluted	22 28	1/5	1/3
				1:2	6/12	—	0/3
				1:4	0/12	—	—
				1:8	0/12	—	—
				1:16	0/6	—	—
		WS	1:640	Undiluted	28/28	2/4	1/4
				1:2	8/12	0/1	—
				1:4	4/12	0/1	—
				1:8	1/12	—	—
		Mak	1:320	Undiluted	12/12	—	—
		Shklyaver	1:320	»	12/12	—	—
		Lee	1:320	»	8/10	—	—
		Chad	1:120	»	2/12	—	—
	A	PR8	1:1 440	1:80	40/40	0/4	0/4
				1:100	38/40	—	—
				1:160	4/10	—	—
Lung suspensions		WS	1:320	1:80	17/18	0/2	1/4
				1:100	14/18	—	—
				1:120	10/12	—	—
				1:160	2/6	—	—
		M15	1:1280	1:100	8/12	1/5	0/2
				1:120	3/12	—	—
				1:160	0/12	—	—
	B	Lee	1:320	1:100	12/18	—	—
				1:160	0/4	—	—

Key. The denominator represents the number of animals used in the experiments; the numerator represents the number of animals which died; - experiment not performed.

The time of death of the mice and the intensity of the changes in the cornea of the rabbits depend on the doses of virus injected and vary in different strains. In general, the A viruses possess the greatest and the B viruses the

least toxic properties; the A-prime viruses occupy an intermediate position in this respect. Thus, for example, the PR8 (A) strain caused the death of mice after the intravenous injection of an allantoic culture (titer of 1:1040, according to hemagglutination reaction) in a dilution of 1:2; after the injection of a lung suspension (titer of 1:1440 according to the hemagglutination reaction), in a dilution of 1:160; the Lee (B) strain gave a corresponding effect after the injection of an allantoic culture (titer, according to hemagglutination reaction, of 1:320) in the undiluted form; and the lung suspension (titer, according to hemagglutination reaction, of 1:320), in a dilution of 1:100; thereby, as a result of the injection of A virus death of the animals occurred in the course of two days; as a result of injection of the B virus, in three days. Allantoic cultures of the PR8 virus produced keratitis in rabbits in a dilution of 1:8; the Lee virus, in the undiluted form.

The toxic effect was not associated with virus multiplication in the bodies of the animals; for example, the period of the greatest death rate of the mice was associated with the time of maximum destruction of the virus in the organism. The same thing applies to experiments on rabbits and guinea pigs. This fact has been shown distinctly by Zakstel'skaya (1953), who determined the concentration of active virus in the blood and organs of mice after the intravenous injection of virus-containing material (Fig. 135).

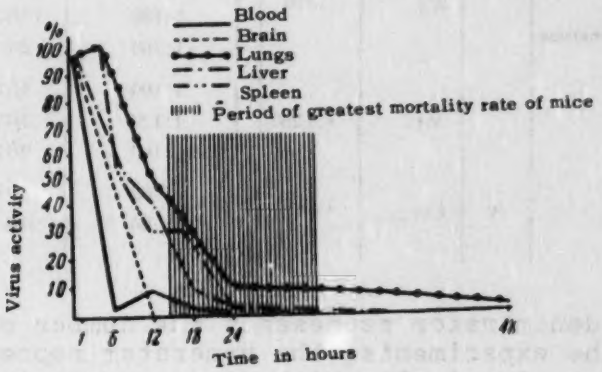

Fig. 135. Concentration of the Active Virus in the Blood and Organs of Mice After the Intravenous Injection of the Virus (Zakstel'skaya, 1953).

The toxic effect is demonstrated by means of tissue-

constriction reaction after Tarusov. Strains of the A viruses possess the most pronounced effect.

The investigations of Zakstel'skaya (1950, 1951, 1953) showed that the toxic properties of allantoic cultures and mouse lung suspensions are most pronounced before 24 hours, that is, at a time when the greatest quantity of active virus is contained in the allantoic fluid and lungs of the mice (Figs. 136, 137). The toxic factor may be found also in the blood of people sick with influenza in the acute stage of the disease.

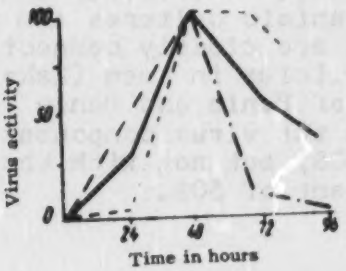

Fig. 136. Change in the Infective and Toxic Properties of the Allantoic Fluid Depending on the Time It Is Taken from the Chick Embryo.

Key: --- change of the agglutinating properties; change in the toxic properties; -.-.-. change in the infectious properties (Zakstel'skaya, 1953).

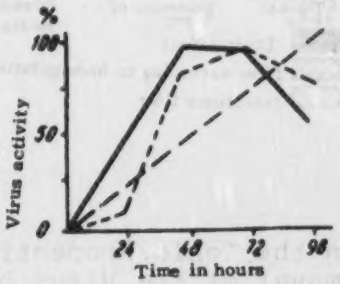

Fig. 137. Concentration of Virus in Lung Tissue of Mice and Change in the Toxic Properties of It Depending on the Time of the Investigation.

Key: ___ intensity of toxic effect; --- pathological changes; _ _ _ concentration of virus according to hemagglutination reaction (Zakstel'skaya, 1953).

On heating the allantoic virus culture the toxic and

infectious properties are inactivated simultaneously (at 56°), whereas the hemagglutinating properties are preserved even at higher temperatures (60°). A similar phenomenon is observed also after the effect of sunlight, intense ultraviolet irradiation and formalin on the virus. After prolonged standing in the cold (4-6°) the infectious properties disappear earliest (after ten days), and then the toxic (notable decrease after 20 days) properties, and later, the hemagglutinating properties. In experiments with centrifugation and adsorption the toxic properties were found to be inseparable from the infectious properties of the active virus (Figs. 138, 139). Therefore, the capacity for the toxic effect of allantoic cultures and lung suspensions of the influenza virus are closely connected with the presence of active virus particles in them (Zakstel'skaya, 1953). In the experiments of Henle and Henle (1946) the toxicity was associated with the virus component having a sedimentation constant of 600S, but not with the one having the sedimentation constant of 30S.

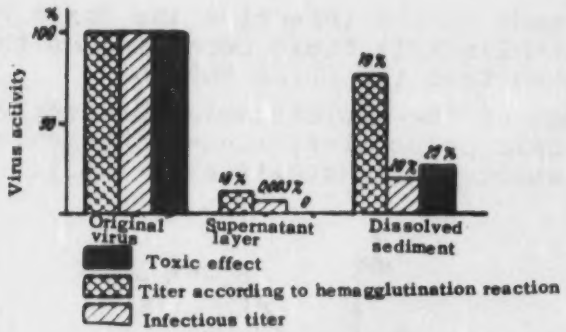

Fig. 138. Change in the Toxic Properties of the Fluid After Removal of the Virus by Centrifugation (Zakstel'skaya, 1953).

The toxic effect of the influenza virus is strictly specific and is neutralized by influenzal antisera in high dilutions against the given virus as well as by sera of people who have had influenza. No cross neutralization reaction is observed between sera of different serological types. Normal rabbit sera do not neutralize the toxic effect of influenza viruses even in low dilutions. Mice immunized by the corresponding virus also acquire a resis-

tance to the practical effect, whereby this resistance is not only species-specific but also strain-specific (Zakstel'skaya). At the same time, intoxication with sublethal doses of the virus (experiments with intracerebral infection of the mice) also involves the development of anti-infectious immunity to influenza (Wagner, 1952).

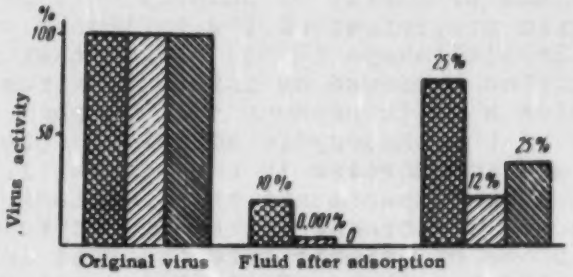

Fig. 139. Change of Toxic Properties of Fluid After Removal of the Virus by Adsorption Onto Erythrocytes.

Key: Same as for Fig. 138 (Zakstel'skaya, 1953).

In experiments on rabbits certain characteristics were found in the reactivity of the animals to the effect of the toxic factor of the influenza viruses (Bennet and others, 1949). If after injection of influenza virus which produces a pyrogenic effect this same virus is injected again after 24 hours the rabbits do not react to it with the development of fever. This resistance to the pyrogenic effect of influenza viruses is maintained for four days, after which it begins to decrease, disappearing by the 11th day. The resistance to the pyrogenic effect of influenza viruses is not associated with antibodies, because it occurs when there are none and, on the other hand, disappears when there are antibodies. The resistance is expressed not only in the absence of fever at the time of the second injection of the virus into the rabbits but also in the absence of other manifestations of the toxic effect, for example, leukopenia. The resistance described here is not specific in the sense of immunity but resembles the phenomena of the so-called "receptor gradient" described in the second chapter: after the injection of PR8 virus the rabbits become resistant to the pyrogenic effect of this virus and to the virus of Newcastle disease but not of the Lee virus. After the injection of the Lee virus resistance develops to the PR8 and Newcastle disease viruses, and the latter virus produces resistance only to itself but not to the influenza viruses. Therefore, the signs of toxicity are associated with the interaction between the virus and

the cell receptors (mucoproteins). Experiments in which the toxic reaction did not develop if inactivated virus or a filtrate of the cholera vibrio was injected into mice intracerebrally two hours before injection of the active virus speak for such an explanation (Fong, Louie and Ching, 1953).

Apparently, the suppression of the protective forces of the body and primarily of phagocytosis is associated with the toxic properties of the influenza virus. In our laboratory Zakstel'skaya (1953) showed that after experimental infection produced by influenza virus strains pathogenic for mice a quite pronounced leukopenia is observed, suppression of the phagocytic activity of the leukocytes in animals and an increase in the permeability of the lung tissue. In these experiments the leukopenia was inevitably demonstrated by a decrease in the leukocyte count in the peripheral blood of animals infected with influenza virus. After isolating a culture of staphylococci from mice which had been well phagocytized by the leukocytes of the animals' blood, Zakstel'skaya showed that after infection of them with the influenza virus the phagocytic activity of the leukocytes falls off sharply. In experiments with the infection of animals with sublethal doses it has been established that in the animals which survive the phagocytic activity is restored to normal. The intranasal introduction of a typhoid agglutinating serum was used as an index of permeability of the lung tissue. Zakstel'skaya showed that in mice infected with influenza virus the permeability of the lung tissue increases markedly in the initial stage of the infection, which is shown by the higher antibody titers to typhoid bacteria after the intranasal inoculation of the serum. Afterwards, with the development of pulmonary hepatization the permeability of the lung tissue decreases. All these properties are expressed in the highly pathogenic strains adapted to mice in serial passages and are not very pronounced (particularly the suppression of phagocytic activity) in strains freshly isolated or passaged in chick embryos.

Apparently, the suppression of the hematopoietic system and of the phagocytic activity is associated with the toxic properties of the influenza virus, and the increase in tissue permeability is associated with the destruction of the mucoproteins of the lung tissue by the virus.

In this section it is expedient also to analyze the observations made on the pathogenicity of influenza viruses which are not transferable in a series of passages.

Sugg (1949, 1950) observed the development of a fatal pneumonia in mice after infection of them intranasally with

allantoic fluid of an influenza A-prime (CAM) virus strain not adapted to them. Fatal pneumonia developed only after the injection of massive doses of the virus; and the virus thereby was not transferable to the mice in serial passages. The pathogenesis of this phenomenon was explained by the toxic extracellular effect of the virus and the adsorption of it onto the surface of sensitive cells but not as the result of the intracellular multiplication in the first passage.

Gorbunova (1953) made a special study of this problem in our laboratory.

The study of the pathogenicity for mice showed that freshly isolated strains of the B virus (Kri, S8, Laz and Ts) possessed different properties after intranasal infection of the mice with allantoic cultures. The pathogenicity was most pronounced in the Kri strain; the S8 and Laz strains were less pathogenic; embryonic cultures of the Ts strain were not pathogenic in any of the experiments.

The pathogenicity of the new B virus strains for mice was determined by the usual method of intranasal infection under light ether anesthesia. The allantoic fluid of the seventh egg passage of the Kri strain proved to be pathogenic for mice in a dilution of 10^{-4}. However, an attempt to passage such an active virus in mice proved to be unsuccessful. The intranasal inoculation of both the concentrated and diluted suspension of mouse lungs did not produce any reaction; all the mice remained alive for 10 days, and at autopsy no signs of pulmonary pathology were found in them. The serial implantations proved to be unsuccessful in six successive passages. The method of Sekretta (1947), Kravchenko and Sekretta (1950), consisting of the repeated intranasal introduction of non-lethal doses of the virus was used for best adaptation; the Jones method was also used (1950).

On study it was found that the virus possessed properties of pathogenicity for mice. The pathogenicity was expressed during the first few intranasal passages of the allantoic fluid without any adaptation. At the same time, experiments of serial passages in mice were negative, that is, after intranasal infection it was not only impossible to produce death of the mice in the second passage but it was impossible also to find any pulmonary pathology in mice killed 10 days after the infection.

Very similar results were obtained in experiments with other strains (Sz, Laz, Ts) of the B virus.

In analyzing the results obtained in experiments with the Kri strain, we can not explain the solid hepatization of lung tissue in mice as the effect of the toxin for the following reasons. First, pneumonia in mice was

accompanied by the accumulation of hemagglutinins which could be identified with the influenza type B virus in the hemagglutination-inhibition reaction; secondly, pneumonia was found after intranasal infection of a very dilute allantoic fluid; thirdly, experiments with the infection of a suspension of lung tissue inactive for mice, taken from the first mouse passage of the Kri and S8 strains, into the amniotic cavity of the embryo constitute proof of the existence of the live virus capable of multiplication in the embryo. These experiments are particularly demonstrative, because the lungs affected by the virus had been preserved about one month in the frozen state (minus 20°) before the new passage was made in embryos. The lines of the Kri and S8 strains mentioned were carried repeatedly through embryos after a single mouse passage. However, the properties of the allantoic virus of the Kri strain which had undergone 13, 19 and 31 egg passages after the single mouse passage and of the S8 strain which had undergone two or three egg passages after the mouse passage were unchanged with respect to the pathogenic pneumotropism shown in the first few passages in mice. They remained just as non-transferable as had the initial allantoic cultures which had never been passaged through the bodies of mice.

The data obtained can not be considered the expression of a toxic effect either, because the toxic properties are most pronounced in strains adapted to mice. Without returning to this problem, which was analyzed in the previous chapter, we should like to note only that it has been shown through the experiments of Zakstel'skaya (1950) and Marennikova (1952) that only influenza A-prime virus strains which had been adapted to mice (Gavr, YeM, Klim) possessed pronounced toxic properties for mice. As one of the expressions of the pathogenic properties the toxicity has a species specificity (although less pronounced than the other expressions of pathogenicity) and is directed at overcoming the defensive forces of the body of the species to which the given virus has been adapted.

It seems to us, therefore, most correct to treat the data obtained as the production of an incomplete virus similar to what occurs after the infection of a chick embryo with massive doses of the influenza virus.

42. Metabolism in Experimental Influenzal Infection

At the present time only separate divisions of metabolism have been studied in influenza, and it is still impossible to present a total picture of the changes in the metabolism of the body as a whole or of its tissues or cells

which occur as a result of the development of the influenzal infection.

The first stage of development of the influenzal infection, which represents the result of contact of the virus with susceptible cells, has been the most studied. It has been shown above that the essence of the developing reaction is a destruction of the mucoproteins of the surface (membrane) of the cells by the virus. There is no need to repeat everything which has been presented in the second and this chapter; it is necessary only to point out that the process of destruction of the mucoproteins by the influenza virus enzyme consists not only in a dismembering of the glucosamine grouping but also apparently in a destruction of the polypeptide linkages. Curtain's (1953) observations which showed that the influenza virus enzyme acts on the terminal amino group of lysine and, therefore, is not only a mucinase but also a peptidase.

After the penetration of the influenza virus into susceptible cells various metabolic disturbances develop in the appropriate tissues. The disturbances in the carbohydrate metabolism have been studied in greatest detail, whereby the main investigations in this direction have been carried out by Tovarnitskiy and his colleagues.

Cheburkina (1950), through a model of a developing chick embryo infected with influenza virus, showed that during the course of the infection marked disturbances in the metabolism of the phosphorus- and nitrogen-containing substances occur. The virus multiplies on the strength of the respective compounds of the cell. However, the changes produced by absorption of these substances by the virus itself are insignificant in the general balance of the cell and can not explain the pathological phenomena observed.

In the next work Cheburkina and Zakstel'skaya (1950, 1953) studied the phosphorus metabolism in the chick embryo infected by influenza virus dynamically; they showed that during the course of development of the infected embryo the accumulation of small quantities of insoluble phosphorus occurs belonging to the group of virus nucleoproteins. In the allantoic fluid the quantity of soluble phosphates and of soluble nitrogen compounds decreases markedly. Apparently, the energy of the phosphorus compounds is used for the synthesis of virus nucleoproteins. In discussing the study of rates of the metabolic processes, the authors obtained data which showed that with the development of the influenzal infection the permeability of the allantoic membrane for phosphorus is increased; the rate and degree of phosphorus uptake in the composition of phosphates of the infected embryo is also increased, whereby the age of the embryos has an influence on the rate of uptake.

Vorob'yeva (1953, 1954) studied the influence of adenosinetriphosphoric acid on the multiplication of the influenza virus in developing chick embryos, in tissue culture, in deembryonated eggs with the presence of inhibitors and without them. Usually, the addition of adenosinetriphosphoric acid to the allantoic cavity, tissue cultures and deembryonated eggs was accompanied by an increase in the biological titer of the virus by one logarithm.

If sodium fluoride or malonic acid and dinitrophenol, which, as is well known, inhibit the resynthesis of adenosinetriphosphoric acid, are added to the culture the yield of the virus is markedly reduced. The addition of adenosinetriphosphoric acid partially eliminated this inhibition. These experiments enabled the author to come to the conclusion that in the synthesis of substances included in the composition of the virus the energy of adenosinetriphosphoric acid is utilized.

The significance of adenosinetriphosphoric acid in the multiplication of the influenza virus was studied again in detail by Vorob'yeva (1954). After the injection of the preparation into the allantoic cavity of chick embryos infected with influenza A-prime (S6) virus the titer of the latter rose from 10^{-8} (control) to 10^{-9}, and in certain experiments, up to 10^{-10} to 10^{-12}. In experiments with the multiplication of the virus in tissue cultures, where the concentration of adenosinetriphosphoric acid is less than in the chick embryos the addition of it increased the accumulation of virus by a 100-1000 times. Similar data have been obtained in cultivating the virus in deembryonated eggs by other authors as well.

The virus requires inorganic phosphorus which is found in the embryonic fluids. After the injection of radioactive phosphorus into the allantoic cavity the latter can be found as part of the virus particles: about 20-25 percent of it is contained in the virus in the form of phospholipoid; the rest is found in the virus protein and, probably, in the nucleoprotein. After the treatment of the virus with ether and the division of it into two fractions -- hemagglutinating and complement-fixing, which represent the soluble antigen, the radioactive phosphorus is found only in the latter fraction (Hoyle, Jolles and Mitchell, 1954).

Glucose is needed for the multiplication of influenza viruses. The addition of glucose to the deembryonated eggs markedly increases the yield of virus, which occurs in parallel with the increase in oxygen consumption (Daniels, Eaton and Perry, 1952).

The relationship of the influenza virus multiplication to the Krebs cycle has been studied by Ackermann and

Kleinschmidt (1951). In experiments of cultivating the influenza virus on the chorioallantoic membrane in the Warburg apparatus it was shown that the replacement of oxygen by nitrogen stops the multiplication of the virus. Malonate, which stops the oxygen consumption of the tissue, possesses the same effect. Therefore, the normal carbohydrate metabolism of the tissue, or, more accurately, the stage of conversion of citric acid is necessary for the multiplication of the influenza virus.

In the experiments of Cheburkina and Zakstel'skaya (1953) data were also obtained concerning the absence of any difference in the concentration of the phosphorus in nucleic acids of infected and control embryos. The exclusion of the specific activity of insoluble phosphates of the embryonic tissue indicates that in influenzal infection an increase apparently occurs in the rate of intramolecular metabolism of nucleic acids in the body of the embryo, whereas the quantitative aspect of the intermolecular metabolism of the latter is unchanged.

Multiplication of the virus in the chick embryo is accompanied by an increase in the consumption of amino acids, which can be explained by an increase in the metabolism of the embryo itself (Ende, 1950). The relationship of the concentration of different amino acids in the chick embryo (Kalter, 1950) is changed. Methionine is absolutely essential for the multiplication of the influenza virus (Ackermann and Kleinschmidt, 1951).

Hyaluronidase does not affect the growth of the virus in the embryo (Fastier, 1951). Cortisone increases the yield of A and B viruses, and the weight of the embryos is reduced at the same time compared with the controls (Kilbourne and Horsfall, 1951; Kilbourne and Tateko, 1953).

The growth of the virus is accompanied by an irregular increase in the consumption of oxygen by the egg, which is observed also after the injection of moderate doses of the heated virus; large doses of the latter act toxically, reducing the oxygen consumption (Blumenthal and others, 1950).

Certain energy relationships of influenza virus multiplication in chick embryos have been studied by Ackermann and Johnson (1953). After the injection of 2,4-dinitrophenol, which does not possess any viricidal properties and does not affect synthesis, the virus multiplication was inhibited, because the respirations and phosphorus consumption by the tissues was markedly stimulated. This gives us the grounds for the belief that energy for the synthesis of the virus is taken from the oxidative and phosphorylating activity of the tissues. The reaction of the allantoic fluid during the process of virus multiplication becomes

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more acid, and the reduction in the pH to lower than 6 leads to a cessation of virus multiplication (Fauconnier, 1953).

A detailed study of metabolism in experimental influenzal infection in mice has been carried out by Volukskaya (1953, 1954). After the infection of mice with pathogenic strains of the influenza virus and the development in them of influenzal pneumonia the wet weight of the lungs increased by 20-40 percent or more, which was not observed after infection of the mice with non-pathogenic strains of the virus. At the same time, an increase also occurred in the dry weight of the lung by 20-30 percent or more. In contrast to this, the wet and dry weights of the liver decreased by an average of 20 percent. In the lungs of the mice a regular increase occurred in the sugar concentration (by 40-50 percent) and at the same time the concentration of glycogen in the liver (by 20-30 percent) and in the blood (by 20-40 percent) decreased. For the purpose of solving the problem of whether the influenza virus produces changes in the redistribution of sugar in the body or whether it is nonspecific and associated with the development of pneumonia, experiments were performed with the injection of 50 percent ammonium chloride into the repiratory tract. Despite the fact that pneumonia developed thereby which was morphologically similar to influenza pneumonia, a redistribution of sugar in the body did not occur. It did not occur either in asymptomatic influenzal infection.

The study of the enzymes in the bodies of mice showed that the activity of the blood amylase decreases slightly in infections; the activity of the pancreatic amylase increases by 50 percent; the activity of the liver phosphorylase increases somewhat. Since all these changes are observed only in infection with virulent strains, the author concludes that the disturbances in carbohydrate metabolism are associated with a toxic effect of the virus.

Khazanov and Marennikova (1954) also compared the changes in metabolism of mice in asymptomatic and manifest influenzal infection. According to their data, asymptomatic influenzal infection associated with multiplication of a non-pathogenic variant of the influenza virus strain in the lung tissues of the mice did not produce any considerable changes in the total quantity of nitrogen- and phosphorus-containing substances or in their acid-soluble or acid-insoluble compounds. In overt influenzal infection accompanied by morphological changes in the lungs a considerable increase in the wet and dry weights of the lung tissues as well as of the accumulation of nitrogen- and phosphorus-containing substances was observed by 48 hours after the

infection of mice with a pathogenic variant of the same influenza virus strain. The increase in the nitrogen- and phosphorus-containing substances in the lungs occurs chiefly through the accumulation of acid-insoluble compounds.

The investigations made led the authors to the conclusion that the pathogenic effect of the viruses determined not only by its multiplication in the body but also by its other properties, primarily by toxic properties which produced disturbances in the physiological functions of the body.

The data of Vorob'yeva (1954) are in accordance, in general, with this conclusion; in experiments on mice infected with avirulent A-prime (S6) virus, she found a reduction in the concentration of adenosinetriphosphoric acid as a result of its consumption by the virus, which was even more pronounced after infection of the mice with A-prime virus strains which were virulent for them (YeM-prime, Klim-prime) and A (PR8).

Metabolism in influenzal infection was also studied with the application of the method of virus cultivation in mouse tumors. According to the data of Gorelov (1950), the multiplication of the influenza virus in a malignant tumor is accompanied by a reduction in the total quantity of amino acids without any change in the relationship between the thymo- and ribonucleic acids. However, the metabolic rate of nucleic acids is increased in the infection (Rivkind, 1950). Here, the quantity of low-dispersion proteins of the albumin type is reduced, and the quantity of globulins as well as the nonprotein nitrogen are somewhat increased (Gorelov, 1950). The concentration of glucose and of glycogen is not reduced (Gorelov, 1950).

In summarizing the results of these investigations, Tovarnitskiy (1953) comes to the conclusion that the multiplication of the virus itself in the affected organism is not the cause of the disease.

The cause of pathogenicity of the viruses does not consist in the fact that they deprive the body of a large quantity of nitrogen- and phosphorus-containing compounds during the course of their multiplication, because the quantity of these substances utilized by the virus for constructing its own body is negligible compared with the total balance of them in the body. The cause of pathogenicity of the viruses consists in their general effect on the body. This effect amounts to a marked disturbance in the normal course of the metabolic process of the total affected organism. The mechanism of this effect has not as yet been studied, because many functions of the viruses are still unknown. It may be supposed only that in infection by the influenza virus the essential role in its pathogenic effect

may belong to the toxic factor. The data obtained permit us to come to the conclusion that the pathological process in influenza is basically a total, generalized process which involves many functions of the central nervous system and internal organs, and that the principal part in the pathogenesis of the infection belongs to the toxic effect of the influenza virus.

The energy relationships during the course of reaction of the influenza virus with the lung tissue of mice have been studied by Tovarnitskiy (1951), Tovarnitskiy and Lutikova (1949, 1951), Lutikova (1950, 1951).

The reaction of the virus with the lung tissue is exothermic and is accompanied by mitogenetic radiation (ultra-violet rays in the short and middle portion of the spectrum). By means of a yeast detector it is possible to show a considerable mitogenetic effect from the contact of the virus with the lung tissue of the mice. The strength of the effect depends on the dose of the virus: with 5000 MLD it is equal to 39-49 percent; with 100 MLD, 10-15 percent. The source of the virus, allantoic fluid or mouse lungs, does not play any part in this. The mitogenetic effect is extinguished by type-specific influenza antiserum (Lutikova, 1950) as well as after blockage of the virus (its free functional amine and carboxyl groups) by papain and cytochrome. The chemical reactions from the interaction of the virus with the cells proceed rapidly and are completed in five minutes (Tovarnitskiy, Lutikova, 1949). The effect of radiation is not produced by the hemagglutinin but by the biologically active virus, because after heating the virus it disappears, although the heated virus still preserves its hemagglutinating activity. Various strains of the virus are characterized by an unequal degree of radiation activity in their reaction with susceptible tissues: it is greater in the A viruses than in the B virus; greater in mouse-adapted than in freshly isolated strains. Therefore, the intensity of the radiation effect depends on the multiplication activity of the virus (Lutikova, 1951).

According to the data of Tovarnitskiy (1951), the process of the reaction of the influenza virus with susceptible cells is exothermic and is accompanied by ultraviolet radiation. The reaction occurs with great rapidity and is completed in five minutes. It is of a quantitative nature (depends on the titer of the virus), is strictly specific (neutralized by specific serum) and possesses a definite adaptive mechanism (it occurs only when the virus reacts with a susceptible cell).

Certain functional groups of cell protein (carboxyl and amine) play an essential part in this reaction. Blockage of them with antibodies or chemical agents prevents the

reaction of the virus with them.

Yeremeyeva's (1953) assertion that the influenza virus penetrates into the cell without disturbing its physiological functions and without a chemical (enzymic) reaction with its surface components does not have much basis, therefore. The enzymic reaction is an initial phase in the influenzal infection.

Afterwards, with the reaction of the virus and the susceptible cell (activity of the virus within the cell), the virus substantially disturbs its metabolism and utilizes particularly the macroergic linkages of the cell polyphosphates as sources of energy for multiplication or for the synthesis of virus nucleoprotein.

43. The Effect of Environmental Factors and the Role of the Nervous System in Infection

The effect of the environmental temperature on the course of experimental influenzal infection has been studied repeatedly. Sulkin (1945) put influenza-infected mice into rooms having a temperature of 35° and 15°. The mortality rate of the infected mice did not change much, but in animals kept in a warm room the pulmonary pathology was less pronounced than in those kept in a cold room.

More distinct data were obtained by Zhilkina (1952), who used severer cold stimuli. The experiments were performed on mice and young rats which before the virus infection had been placed in a room having a temperature of -3°, -4° for 10 minutes, and which in other experiments had been subjected to chilling after being kept for five minutes at 37°. The mice which had been chilled died from sublethal doses of the virus.

In the experiments of Kurashvili (1953), after putting mice into a cold room (from 0° to 2°) it was impossible to demonstrate any changes in the concentration of virus in the lungs, although the body temperature of the mice dropped to 30-32°. At the same time, the chilled animals infected with lethal doses of the influenza virus died somewhat earlier than those which had been under the ordinary condition.

Wilson and others (1947) showed that the chilling of monkeys leads to the fact that influenzal infection, which usually occurs asymptomatically, is accompanied by the development of pneumonia.

Therefore, the chilling increases the sensitivity of the animals to the influenza virus and leads to a more severe course of the infection. The effect of radiant energy was studied by Beutler and Gezon (1952). Irradiation of the mice with X-rays in a dose of 200-700 r depressed the elaboration of antibodies to influenza but the

susceptibility, morbidity rate and mortality rate of the animals did not change. In the experiments of Blumenthal and co-authors (1950), X-irradiation of chick embryos infected with the influenza virus prolonged the incubation period and reduced the infectious titer. According to the data of Peterson and Kozlova (1957), guinea pigs irradiated with X-rays (200 r) became susceptible to influenza.

The effect of various factors on the course of influenzal infection in mice was studied in detail by Yefimova (1954). The influence of chlorine, ammonia, benzol, high and low temperature and ultra-violet radiation on the course of influenza was investigated. The effect of these factors was studied in the chamber. A concentration of chlorine in the air of 0.002-0.02 milligram per liter; of ammonia, 0.02-0.1 milligram per liter; of benzol, 0.5-3 milligrams per liter did not exert any kind of noticeable effect on the course of influenzal infection in mice. Keeping the animals at a temperature of 35° and 5° did not affect the development of the infection either, although in the latter case a more pronounced accumulation of the virus in the lungs was observed. Preliminary (before the infection) ultra-violet irradiation had a favorable influence on the development of the infection, reduced the mortality rate of the animals and the accumulation of virus in the lungs. The same results were obtained after ultra-violet irradiation of animals following the infection. The defect of this work is the use of exceedingly large doses of the virus, which do not permit the detection of the influence of certain of the factors studied as a result of the mortality of 70-90 percent of the animals in the control group.

The absence of an influence of chlorine on the development of the influenzal infection in mice was also established by Smorodintsev (1953), who used different concentrations of the virus for infecting the animals and who studied the development of the infectious process dynamically.

Here, we are not analyzing the problem of the disinfectant effect of chlorine and ultra-violet rays on the influenza virus which is in the air, since this has been discussed in the second chapter.

The effect of nutrition on the course of the influenzal infection was also studied by certain investigators. In the experiments of Wilson and others (1947) defective nutrition and particularly a defect in vitamin B in the food of monkeys reduced the resistance of these animals to influenzal infection. Young and co-authors (1949) showed that the removal of vitamin D from the rations of mice increases the susceptibility of them to infection with the

swine influenza virus, particularly in females. Therefore, the course of the experimental influenza is aggravated under the influence of cold and inadequate nutrition (particularly, inadequate vitamins); these factors increase the susceptibility to influenza. On the other hand, the increase in resistance of the animals to influenza under the influence of ultra-violet irradiation has been established.

Kravchenko and Sekretta (1950, 1953) caused the death of mice from sublethal doses of the influenza virus when physiological solution was introduced repeatedly and intranasally on subsequent days. The authors believe that in this case the physiological solution and other substances introduced into mice acted like nonspecific stimuli. However, Smorodintsev and Boychun (1955), repeating these experiments, explain their results by the mechanical generalization of the process as a result of the introduction of fluid into the lung. In subsequent years certain problems have been studied in detail in the USSR concerning the role of the nervous system in the development of the influenzal infection and the effect of the influenza virus on the nervous system.

According to the data of Kurashvili (1952, 1953), drug-induced sleep produced by the injection of anesthetics into mice, changes the course of the experimental infection. Veronal anesthesia inhibits the development of the infection, which is expressed in a lesser degree of accumulation of the virus in the lungs of experimental animals compared with the controls. A three-day sleep lengthened the lives of the animals infected with lethal doses of the virus by one or three days, but with the elimination of the anesthesia the virus began to develop and the animals died. After infection with 5 or 10 LD_{50} of the virus the animals died with equal frequency without anesthesia and with anesthesia. After infection with minimum lethal doses, 50-60 percent of the animals survived in the experimental group.

During the course of subsequent controversies it was made clear that the first stage in the reaction of the virus with the sensitive cell, fixation of the virus onto the cell, occurs in anesthetized mice less actively than in the control animals, and in this respect approaches the indices of virus fixation in immune animals. It has also been shown that in the anesthetized animals the elution of the influenza virus from the sensitive cells is somewhat increased. All these facts can contribute to checking the reproduction of the influenza virus in anesthetized animals.

The reproduction of the virus in the lungs of alcoholized mice was suppressed during the first two or three days after their infection with small doses of the virus.

However, beginning with the third day the virus multiplied unchecked and reached the same numbers as in the control animals. Prolonged use of alcohol (for 10 days) aggravated the course of the influenzal infection of white mice.

In a special series of experiments the author excluded the nerve receptors of the lungs by means of vagotomy, transection of the sympathetic trunk, crushing the superior sympathetic ganglia, and pharmacological procedures (novocain, atropine, sympathin). However, these procedures did not affect the reproduction of the influenza virus in mouse lungs. After acting on the nervous system with stimulants (caffein, ephedrin, amphetamine, morphine, adrenalin, acetylocholine) the influenza virus multiplied in the lungs of white mice with the same intensity as in the control animals, whereby the course and outcome of influenzal infection became more severe in the great majority of animals.

Somewhat different data have been obtained by Korovin (1952), who showed that the intranasal introduction or intravenous injection of 10-percent novocain solution into mice 10 minutes to 24 hours before infection of them with the influenza A virus exerts a distinct prophylactic effect, which is expressed in a lengthening of the periods of survival and an increase in the percentage of surviving mice.

Sokolovskaya and Monastyrskaya (1953) stimulated the superior cervical sympathetic ganglia of mice or crushed them, producing a circulatory disorder in the lungs in the form of an overt hyperemia. If after this the mice were infected with repeated lethal doses of the virus, an inhibition of the virus multiplication was observed in their lungs; however, the death of the animals was just as frequent and occurred at the same time. After infection with sublethal doses or one or two lethal doses no such differences were observed in the accumulation of virus, and the mortality rate was greater among animals which had had trauma of the sympathetic ganglia.

In generalizing these data, Smorodintsev and coauthors (1953) indicate a definite lag in virus reproduction in the lungs of mice anesthetized with veronal (Fig. 140). The greater the stimulating dose, the less pronounced this effect, and it was most pronounced after infection with minimum doses. The inhibition of virus reproduction was greater the longer the anesthesia; however, lengthening it by more than 72 hours leads to the death of the animals from veronal intoxication. After the anesthesia is stopped a rapid multiplication of the virus begins, which culminates in the death of the mice, usually one or three days later than in the control. The suppression of virus multiplication was the greater the earlier the veronal anesthesia was used.

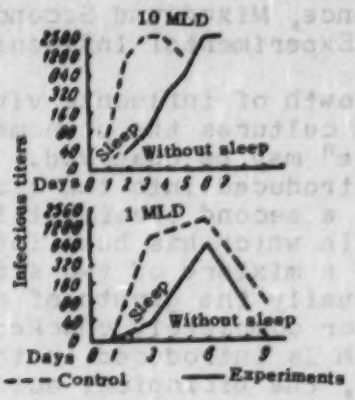

Fig. 140. Effect of Veronal Sleep on the Accumulation of Virus in MLD in the Lungs of Mice (Smorodintsev, 1953).

The effect of the influenza virus on the mechanism of transmission of impulses in the sympathetic ganglia was studied by Medvedev (1953, 1954). The influenza A virus (Shklyaver) was investigated as a stimulus for the superior cervical sympathetic ganglion under conditions of perfusion according to the Bykov method. The experiments were performed on cats. In these experiments it was shown that the influenza virus exerts a toxic effect on the metabolic processes in the sympathetic ganglion. While it does not change the conductivity or excitability in response to an electrical stimulus in low concentrations, it eliminates the humoral transference of the nerve impulse, and in high concentrations changes the excitability and conductivity of the ganglion fibers. The effect of the influenza virus resembles the effect of nicotine. The virus does not act on immunized animals.

Merkulova (1954) showed that the injection of large quantities of the influenza virus into rats, which leads to the development of intoxication, is accompanied by prolonged vegetative disorders (dyspnea, ruffling of the fur, chill), a prolonged disturbance in conditioned-reflex activity and a slow return to normal. The injection of normal allantoic fluid led to considerably less pronounced disorders. The extinction of conditioned-reflex activity with the increase in the severity of the clinical course of influenza in animals has also been shown by Bazyka (1952) in experiments on mice.

44. Interference, Mixed and Secondary Infection in Experimental Influenza

With the growth of influenza viruses in chick embryos or in tissue cultures the phenomena of the so-called "virus interference" may be observed. If first one strain of the virus is introduced into the tissue culture and then, after a day, a second strain, this latter does not develop. The strain which has been introduced first has the advantage. If a mixture of two strains are introduced into a culture, usually the growth of one of them is partially suppressed or completely checked. The strain has the advantage which is introduced in the greater concentration. In addition, the biological activity of the strains is of importance in the phenomena of interference (Andrewes, 1942).

The phenomenon of interference is observed both between various strains of the same virus (Andrewes, 1942) and between different viruses (Ziegler and Horsfall, 1944). The interference may be demonstrated after the infection of chick embryos, mice and rabbits with influenza viruses as well as in tissue cultures.

Interference may be observed between influenza viruses and other viruses -- swine influenza (Ziegler and Horsfall, 1944), yellow fever, West Nile and Venezuela encephalitis (Lennette and Koprowsky, 1946).

Smorodintsev and Chalkina (1953) studied the interference of A and B viruses in mouse lungs, using the neutralization of one of them with type-specific serum for the calculation of the number of viruses in the mixture. The authors showed that the outcome of interference between A and B viruses depends chiefly on the quantitative interrelationships between them at the time of their encounter in the susceptible tissue. The greater the concentration of one of the reacting viruses at the beginning of this encounter the lower the rate of multiplication of the second. With the simultaneous introduction of the same quantities of reacting viruses into the susceptible tissue the more virulent virus multiplied predominantly; in the authors' experiments, the A virus. However, after increas- the infective dose of the B virus by 10 times and leaving the A virus dose the same as before the rates of multiplication of them equalized. In general, the phenomenon of interference between different viruses resembles the phenomena of autointerference described above to a large extent.

The phenomenon of interference, however, does not exclude the development of a mixed infection produced by different influenza viruses as well as by influenza viruses

and other pathogens.

Influenza viruses may be passaged in mixed cultures for a long time. Sugg and Magill (1948) showed that a mixture of A (PR8) and B (Lee) viruses can be passaged in chick embryos and in mouse lungs without eliminating either of the strains from the culture. Liu and Henle (1951) obtained similar data with A and B viruses. Gorbunova (1953) showed that from a mixture of A and A-prime viruses it is possible to isolate both components by means of prolonged passages -- through the use of maximum dilutions (Gorbunova, 1954). This work has been analyzed in the previous chapter. Therefore, the influenza viruses of different species may develop simultaneously in an unlimited number of passages in the bodies of experimental animals.

McClelland (1946) established the fact that the multiplication of the influenza virus and typhus rickettsias is possible simultaneously in chick embryos.

Shubladze and Solov'yev (1950) showed that in a mixed infection produced by influenza A and tick-borne encephalitis viruses a multiplication occurs of both viruses each of which produces the clinical picture characteristic of it. The mortality rate of mice in mixed infection is somewhat greater than in an infection produced by each of the pathogens. In a mixed infection produced by three pathogens (influenza, encephalitis and typhus) all three pathogens developed in the bodies of the animals also, whereby the mixed infection was transferred in a series of successive passages. The addition of a fourth -- bacterial -- pathogen (P. tularense) quickly crowded out the first three pathogens.

In the experiments of Zhdanov and Korenblit (1952) the successive infection of mice with influenza viruses and rickettsias was accompanied by a simultaneous multiplication of both pathogens in the lungs (Table 56).

As seen from Table 56, in mixed infection death of the mice occurred sooner than after infection with the influenza virus alone (the rickettsias were injected in a sublethal dose).

After the preliminary injection of killed rickettsias and subsequent infection of the animals with influenza virus the development of the latter was inhibited (phenomenon of interference). In experiments of mixed infection produced by influenza viruses and the virus of louping ill the simultaneous development of both viruses in the bodies of the animals and a more severe course of the mixed infection was noted, which was expressed in the earlier death of the animals.

These investigations have a direct bearing on the

study of the role of secondary bacterial infection in influenza, to which attention was directed simultaneously with the discovery of the influenza virus.

Table 56

Course of Mixed Infection in Mice After Infection of Them With Influenza A (PR8) Virus and After a Day by the Rickettsiae prowazeki

Group	No of mice	Time of death in days in % of total no. of mice				Average area of pulmonary pathology in % of the entire area of the lungs	Hemagglutination reaction	Rickettsias
		2	3	4	5			
Experimental (influenza virus + rickettsias)	30	47	27	10	16	70	193	+++
Control 1 (influenza virus)	15	33	33	–	7	54	180	–
Control 2 (rickettsias)	15	–	–	–	–	42	0	+++

Shope (1931), studying the virus influenza of swine, showed that secondary bacterial flora are of importance in the pathogenesis of the infection. After being infected with the influenza virus the animal suffers from the disease in a mild form. With the simultaneous injection of the virus and H. influenzae suis the disease has a more severe course, with pulmonary pathology.

This problem has been studied in detail by Smorodintsev and his co-workers (Smorodintsev, Drobyshevskaya and Ostrovskaya, 1938) in experiments with the experimental infection in mice. In their experiments on the study of secondary infections in the lungs of mice infected with the influenza virus in combination with the hemolytic streptococcus, pneumococcus and influenza bacillus, Smorodintsev and his co-workers found that the pneumococci, streptococci and, to a somewhat lesser degree, the influenza bacilli are markedly activated in the lungs affected by the virus, and form foci here with a tendency toward generalization and a transition to a septic process. The course of the processes produced by the microbes depends on the intensity of the preliminary pathology inflicted by the virus: the greater the virus pathology the more pronounced the processes.

Vaccinated mice successfully overcome secondary infections which develop against the background of a simultan-

eous infection with influenza virus. The same thing occurs also after the preliminary injection of them with influenza antiserum (Smorodintsev and others, 1938).

Wilson and co-authors (1947) studied the course of a mixed influenzal and streptococcal infection in monkeys. After the intranasal infection with virus (PR8) alone or with hemolytic streptococcus alone the infection in the monkeys has an asymptomatic course. If the virus is introduced 4 to 17 days after the streptococcal infection the animals develop a disease, and in certain cases a fatal streptococcal sepsis.

In the work of Harford and co-authors (1949) it was shown that in normal mice after the inhalation of pneumococci the latter are destroyed in three hours, which is expressed in a reduction in their number. If the mice are infected with influenza virus, after inhalation of pneumococci the latter multiply actively in the lungs.

In the experiments of Zhdanov and Korenblit (1952) the infection with influenza virus aggravated the course of experimental bacterial infection in mice. After infection of the mice with the mouse typhus bacillus (Salmonella typhi murium) with preliminary injection of the influenza virus the mouse typhus bacilli produced an earlier death of the animals when the influenza infection was as yet hardly manifested (Table 57).

Table 57

Course of Infection in Mice Infected With Influenza Virus and a Day Later With Mouse Typhus Bacteria

Group	No. of mice	Time of death in days				Hemagglutination reaction
		1	2	3	4	
Experimental (influenza virus + mouse typhus bacteria)	25	—	19	64	—	—
Control 1 (mouse typhus bacteria)	15		3	12	—	—
Control 2 (influenza virus)	15	—	—	10	5	+++

In mixed infection produced by the influenza virus and the pertussis bacillus earlier death of mice and more pronounced pulmonary pathology were observed than after each infection separately.

Bordet and Quersin-Thiry (1953) showed that after the intraperitoneal injection of the influenza virus into

guinea pigs the sensitivity of them to the Bacillus influenzae is increased by 10 times; the sensitivity to the colon bacillus and the cholera vibrio are unchanged.

The data presented show that influenzal infection, by producing a suppression of the protective forces of the body, stimulates the development of the conditionally pathogenic flora and leads to a more severe course of other infections produced by pathogenic bacteria and viruses. On the other hand, the latter, in turn, produce a more severe course of the influenzal infection in animals after mixed infection.

45. Pathogenesis of Influenza in Man. Pathological Data

After an ordinary non-fatal course of influenza the pathological changes in people are observed primarily in the upper segment of the respiratory tract.

Kolyaditskaya (1947) showed that in influenza, in contrast to acute catarrhs and other influenza-like diseases, characteristic changes are observed in the cytologic composition of the nasal secretion.

Normally, in healthy people the cellular composition of the nasal secretion is sparse. Neutrophils and squamous epithelial cells are found in it. In the morning there are more of them than in the afternoon.

In influenza, columnar epithelial cells appear in abundance in the impressions from the nasal mucosa in the acute stage of the disease, whereas in acute catarrhs they are considerably fewer.

Malomuzh (1952) studied the histopathological changes in the mucous membrane of the nose in patients with influenza during the outbreak of 1943-1944, whereby he succeeded in taking biopsies of the nasal mucosa in part of the patients (chiefly medical workers) along with the smear-impression. According to the author's data, as early as the first few days of the disease not only hyperemia but also deeper-seated histological changes are observed as early as the first few days of the disease. On the second day of the disease, the ciliated epithelium of the mucous membrane undergoes degenerative changes, and in places sloughs off, exposing the mucous membrane proper (Fig. 141). A round-cell infiltration is observed in the subepithelial layer, most pronounced during the first two or three days of the disease; the mucous glands are in a state of hyperplasia (Fig. 142). On the third or fourth day, the entire mucosa is edematous; the connective-tissue elements are separated by the interstitial basic substances (Fig. 143). Beginning with the fifth or sixth day, a large number of eosinophils and inflammatory mast cells (Fig. 144) appear

in the subepithelial layer, and reparative processes in the mucous membrane of the nose develop (Fig. 145) which are completed during the course of the next week.

Fig. 141. Nasal Mucosa on the Second Day of Influenza.

The epithelium is desquamated in places. In the submucosal layer there is considerable infiltration; the blood vessels are moderately dilated (Malomuzh, 1950).

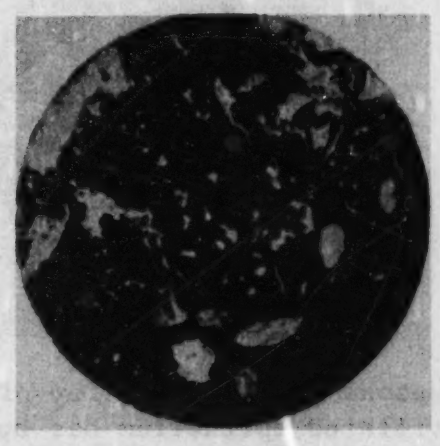

Fig. 142. Nasal Mucosa on the Third Day of Influenza.

Many-layered columnar ciliated epithelium has been desquamated in places. In the subepithelial layer there is a round-cell infiltration. The vessels are dilated and their walls are thickened (Malomuzh, 1950).

Fig. 143. Nasal Mucosa on the Fourth Day of the Disease. The entire mucosa is impregnated with blood elements with the predominance of granular leukocytes. The mucosa is edematous and the mucous glands are in a condition of hypersecretion (Malomuzh, 1950).

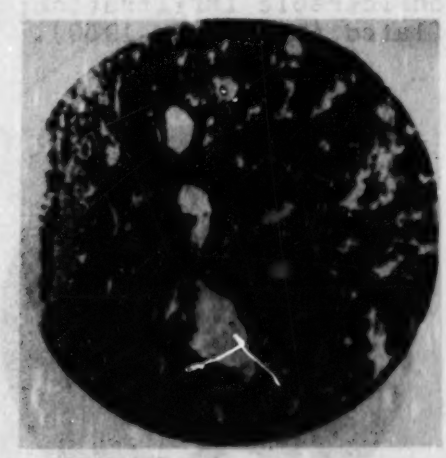

Fig. 144. Nasal Mucosa on the Fifth Day of Influenza. The multilayered ciliated epithelium has been loosened in places and sloughed off. Infiltration of the subepithelial layer with areolar connective tissue has occurred. There are a large number of dilated blood vessels. The glands are alveolar in shape with a predominance of mucous cells (Malomuzh, 1950).

Fig. 145. Nasal Mucosa on the Sixth Day of the Disease. Epithelium is found throughout. The subepithelial layer is in a state of edema and discontinuity. The glands are in a state of normal secretion (Malomuzh, 1950).

In the examination of the upper respiratory tracts of people who have died from influenza similar changes are found.

According to the data of Straub and Mulder (1948), the pathology of the tracheal and bronchial epithelium in patients who died from influenzal pneumonia are identical with those which are observed in mice and ferrets. The surface ciliated columnar epithelial cells become necrotic as do also the deeper cuboidal cells. The basal cells remain but degenerate into squamous cells. With rare exceptions, the epithelium of the bronchioles does not change. Herns and Mulder (1951) point out that if a smaear is made from the mucosal epithelium of the trachea and bronchi of people who died from influenza it is impossible to find any ciliated columnar epithelial cells or they can be found in a negligible quantity. They are completely or partially replaced by undifferentiated or necrobiotic epithelium. In conditions other than influenza the normal structure of the multilayered columnar ciliated epithelium is found on histological examination, but in influenza only several layers of basal cells (undifferentiated) remain, and no columnar cells are seen. These statements by Herns and Mulder do not agree with the data of Afanas'yeva (1954), who found columnar epithelial cells in impressions made at autopsy from the mucous membrane of the trachea and bronchi of children who died from influenza.

In children who died from toxic influenza and influenzal pneumonia considerable changes are found in the motor nerve endings in the muscle apparatus of the larynx. These changes have the nature of a marked irritation -- the preterminal branches look thickened (Fig. 146). These changes are evidently associated with the general intoxication and

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increased functional strain which falls to the lot of the laryngeal muscles in labored respiration (Tret'yakova, 1952). The nerve cells and nerve fibers of the mucous membrane of the nose and nasal sinuses thereby undergo reactive and dystrophic changes also. Changes in the nerve fibers innervating the blood vessels and changes in the ganglion cells are particularly characteristic of influenza, which is evidently explained by the dilatation of the blood vessels and foci of hemorrhages observed in the severe forms of influenza (Zadorova, 1952).

Fig. 146. Changes Occurring in the Motor Nerve Endings of the M. Vocalis of a Child Aged 2 1/2 Months, Who Died From Pneumonia Which Became Abscessed (Tret'yakova, 1952).

Influenza has a course like a general disease with a pronounced intoxication, and therefore pathological changes occur not only at the site of virus multiplication (upper respiratory tract) but also in other organs and systems.

Even during the pandemic of 1889-1890 observations were made which indicated the severe pathology of the vascular system in influenza -- dilatation of the capillaries, necrosis of their walls, hemorrhages into the surrounding tissues (Kuskov, 1893).

Numerous pathological observations have been made in the Soviet Union and abroad during the influenza pandemic of 1918-1919. Lesions during the influenza pandemic of 1918-1919 were characterized by the following description. The inflammatory lesions in the lungs had the nature of a lobular bronchopneumonia with hemorrhages, which were

multiple with a tendency toward confluence of the foci and a shift of the inflammatory process to the pleura. At autopsy, the pathology was variegated, which depended on the different times of development of the various inflammatory-hemorrhagic foci. Exudate in the alveoli was catarrhal (serous fluid rich in red blood cells and poor in fibrin); in the later stages of the disease, it was catarrhal-purulent (with the predominance of polynuclear leukocytes). A tendency of the process to change into suppuration, necrosis and gangrene was noted. In the pleura numerous punctate and larger hemorrhages were observed, and afterwards a seropurulent pleurisy developed. In the upper respiratory tract there were marked acute catarrhal signs, laryngeal edema, and sometimes a croupous laryngitis (Glinchikov, 1922; Kulesha, 1918; Pozhariskiy, 1921).

At autopsy, the heart was enlarged because of a dilatation of the cavities (particularly the right); the muscle was flabby, dull, yellowish or greyish in color, with punctate hemorrhages under the epicardium. On microscopic examination a fatty infiltration of the muscle fibers, diffuse granular decomposition of them, loss of the cytoplasmic structure and nuclear degeneration, and edema of the interstitial tissue were noted, that is, signs of an acute myocarditis (Sysoyev, 1919).

Of the parenchymatous organs, the liver and spleen suffered most. The liver was enlarged, soft on section, with an indistinct picture. Histologically, an albuminoid degeneration, foci of necrosis and hemorrhage were found. The spleen was also enlarged, hyperemic and hyperplastic; sometimes hemorrhagic foci were observed. In the kidneys an albuminoid degeneration, foci of necrosis and hemorrhages were observed at times (Kulesha, 1918; Sysoyev, 1919).

As far as the gastrointestinal tract was concerned, usually signs of an acute catarrhal and sometimes hemorrhagic enterocolitis were observed (Glinchikov, 1922).

The lymphatic apparatus, particularly the lymph nodes in the area of the neck and lungs, was altered; hyperplasia and foci of hemorrhages were observed (Pozhariskiy, 1921).

In the nervous system hyperemia of the pia mater and foci of hemorrhages in it, hyperemia and edema of the cerebral tissue, foci of punctate hemorrhages, and not uncommonly, signs of vascular encephalitis (Pozhariskiy, 1921) were noted.

Similar data are presented also in the works of foreign pathologists (Schmorl, 1918; Goodpasture, 1919; Winternitz and others, 1920; Oberndörfer, 1918).

Later investigations made it possible to show a similar picture.

Scadding (1937) found inflammation of the trachea and

bronchi, necrosis and sloughing of the epithelium, necrosis of the alveolar wall, hemorrhages and inflammatory infiltration of the interstitial tissue on autopsy of those who died from influenza during the epidemic of 1936-1937. As a whole, a diffuse and interstitial pneumonia developed.

According to Abrikosov (1941), the changes in the repiratory organs may be different and consist of a catarrhal laryngotracheobronchitis, and sometimes a hemorrhagic-necrotic or fibrous-necrotic laryngotracheobronchitis. The following changes can be observed in the nose and in the nasopharynx: from hyperemia and hemorrhages to necrosis and ulceration of the mucosa. The lungs are congested. Hemorrhages occur into the parenchyma of the lungs and sometimes serous-hemorrhagic pneumonia.

On autopsy of people who died from primary influenzal hemorrhagic pneumonia (from pneumonia of the "Spanish 'flu" type), an acute catarrhal-hemorrhagic inflammation of the larynx, trachea and bronchi is found. The mucous membrane is edematous, markedly hyperemic with hemorrhages, and is covered with a bloody and sometimes sanguinopurulent mucus. Necrosis, epithelial desquamation, ulceration with fibrous deposits are observed. In the lungs there are serous-hemorrhagic, fibrous-hemorrhagic or simply hemorrhagic pneumonic foci (Abrikosov, 1941; Davydovskiy, 1939; and others). Essentially, this is the picture which was observed in white mice when they were infected with influenza virus. In the latter, influenza proceeds in the form of hemorrhagic pneumonia, at times completely free of microbial flora.

If the disease drags on somewhat, the most varied picture of changes in the lungs may be found at autopsy in the presence of pulmonary complications: catarrhal bronchopneumonia or multiple foci of catarrhal bronchopneumonia of the usual type, sometimes confluent catarrhal pneumonia, fibrinous or fibrino-hemorrhagic pneumonia, purulent pneumonia, gangrene of the lung, degenerative changes in the parenchymatous organs. The pneumonias may be complicated, in their turn, by various pleurisies up to the point of empyema. Purulent lung processes may lead to septicemia with suppurations in various organs. In the presence of other complications aside from the pulmonary ones there are corresponding changes depending on the affected organ and the nature of the complication. Degenerative changes are observed in the parenchymatous organs.

In the heart myocarditis may be observed, according to Abrikosov. It is interesting to note that these observations by Abrikosov have been confirmed virologically by Finland and co-authors (1945) who succeeded in isolating type A influenza virus from the lungs in two cases of

hemorrhagic pneumonia in which myocarditides were also observed.

At autopsy (description of autopsies with which we have had a chance to become familiar) edema and congestion of the brain and meninges, congestion reaching the point of stasis, focal hemorrhages and degenerative changes in the ganglion cells may be observed. According to Abrikosov, sometimes signs of influenzal encephalitis occur which are expressed in the formation of ring hemorrhages in the white matter with a subsequent nodular proliferation of the neuroglia; in other cases, the encephalitis affects chiefly the gray matter of the brain stem area and the gray nuclei of the base of the brain and is expressed in lymphoid and plasma-cell infiltrates in the form of cuffs around the blood vessels, small hemorrhages and a nodular neurological reaction around the capillaries and degenerating nerve cells. Similar changes in the cerebral cortex of people who have died from influenza have been noted repeatedly by a number of other authors (for example, Davydovskiy, Pozhariskiy, Kuskov), who observed hyperemia and edema of the brain, sometimes punctate or more pronounced hemorrhages.

On autopsy of people (young children in the great majority of cases) who died on the third or fourth day of the disease from a fulminating influenza, both sporadic and at the time of the small outbreak in the winter of 1951, edema of the brain, congestion reaching the point of stasis, and sometimes degenerative changes in the ganglion nerve cells were found.

The vegetative nervous system suffers considerably in influenza (Abrikosov), 1941; Mogil'nitskiy, 1941). According to Abrikosov, changes in the sympathetic ganglia are very often observed in the form of degenerative changes in the nerve cells and infiltrates in their stroma composed of lymphoid and plasma cells. According to Mogil'nitskiy, influenza in 82 percent of cases produces greater or lesser changes in the vegetative nervous system. The most pronounced changes are observed in the cervical sympathetic ganglia; lesser changes, in the trunk of the vagus nerve. in the sympathetic ganglia injection of the blood vessels of the interstitial tissue may be found. The interstitial tissue is edematous with perivascular round-cell infiltration, with a location of the leukocytes along the periphery in the blood vessels. Destructive processes occur in the cytoplasm and nuclei of many ganglion cells.

Therefore, the pathological data show that in influenza local phenomena in the respiratory tracts of a necrotic-inflammatory nature are combined with general manifestations of intoxication.

46. Functional Disturbances

In the present section only a part of the data pertaining to the problem being discussed will be presented, since the principal material on the disturbances in the functions of the human body will be described in the chapter on the clinical aspects of influenza.

In addition to a disturbance in the functions of the respiratory organs, in which multiplication of the influenza virus occurs, its activity is expressed throughout the body. A suppression of the nervous system activity, with which the normal functioning of all organs and systems is associated, and a suppression of the protective forces of the body, which constitute a characteristic feature of the traumatic effect of the influenza virus, are of particular importance.

Research done on volunteers in whom the influenza virus was introduced into the respiratory passages gives us some evidence as to the nature of the functional disturbances. The most exhaustive experiments have been performed in his time by Smorodintsev and co-authors (1937) and Henle and co-authors (1946).

Smorodintsev introduced 0.1-1 cubic centimeter of Mouse A virus in the form of a 10-percent lung suspension, which corresponded to approximately 1,000,000 mouse lethal doses, into volunteers by means of inhalation (a total of 72 persons were investigated). About 20 percent of the volunteers became sick with influenza in the mild form with clinical symptoms characteristic of influenza and a hematologic picture (leukopenia, neutropenia, eosinopenia, lymphocytopenia and a relative monocytosis) found in influenza. Those who did not become sick were chiefly the persons who had high titers of antibodies in the blood. The virus died out in the nasopharynx of those infected during the first one or two days after the infection, and after 10-12 days a pronounced increase in the antibody titer to the virus was observed in the blood.

Henle introduced allantoic cultures of the A and B viruses into volunteers (a total of 65 persons were examined) also by means of inhalation in a quantity of 0.4-0.6 cubic centimeter of undiluted allantoic fluid. An increase in temperature (moderate) and mild influenza-like disorders were observed in 90 percent of the volunteers and lasted no more than one or two days; the nature of the reaction did not depend on the preceding antibody level. The virus could be isolated in less than half of the persons infected; after two weeks, an increase in the antibody titer in the blood was observed in the majority. Intranasal infection was ineffective.

In these experiments it was not possible even once to

reproduce the typical picture of influenza with intoxication. The causes of these failures have become clear only recently, when it was shown that laboratory strains of the influenza virus lose their pathogenic properties for man and the capacity of surviving in his nasopharynx (see third chapter). Therefore, the main data concerning the functional disturbances in the human body in influenza were obtained through observation and study of the patients.

The effect of the influenza virus on the nervous system is varied. Bazyka (1952) showed that in influenza disturbances in the higher nervous activity occur, chiefly in the processes of internal inhibition. The adaptive functions of the nervous system are disturbed (Litvak, 1952), which is not uncommonly associated with the involvement of peripheral nerves and central portions of the nervous system functionally (Davidenkov and others, 1951). Disturbances in the unconditioned reflexes occur (Kul'kova, 1951).

In influenza the disturbances in the vegetative nervous system functions are particularly pronounced. Rozenberg (1931) pointed out the vagotropic effect of the influenza virus, considering that during the course of the disease the development of paresis of the sympathetic nervous system follows stimulation of the vagus. During the period of convalescence the activity of the vagus and sympathetic nerves becomes normal. Davidenkov and co-authors (1951), recognizing the presence of serious disturbances in the vegetative nervous system, believe that the clinical phenomena cannot be reduced simply to the predominance of parasympathetic functions. Apparently, there is a more complex effect of the influenza virus on the central nervous system, including on its higher centers -- the brain.

The disorders of the cardiovascular system are intimately associated with the disturbance in the vegetative nervous system activity. They are manifested in a decrease in blood pressure, chiefly in the venous system (Val'dman, 1937), a non-uniform dilatation of the capillaries (Preobrazhenskiy, 1950), a change in the conduction and rhythm of the heart (Vasil'yev, 1937), a reduction in the cardiac muscle tone (Tushinskiy, 1939). These disturbances are usually not associated with anatomic changes in the cardiac muscle but rather are of a functional nature as a result of a disturbance in the innervation (Leytes, 1951).

In influenza the permeability of the blood vessels is increased, which is established by the method of capillaroscopy and by the Nesterov test (Fadeyeva, 1954).

Disturbances in the activity of the other organs and systems will be described in the chapter concerning the clinical aspects of influenza.

The data of the biochemical study of patients with

influenza are quite contradictory.

The investigations of certain authors (Rossiyskiy, 1942; Erez and Gurevich, 1940; Borevskaya and others, 1940) indicate an increase in the sugar concentration of the blood, which, however, has not been confirmed by the research of other authors (Oppel' and Chulkov, 1936; Davidenkov, 1951; Epshteyn, 1949). As Voluyskaya (1953) correctly indicates, the contradictory data may have depended on the fact that diseases of different natures were included under the diagnosis of influenza.

No clear-cut data have been obtained either in investigations on the concentration of mineral compounds in the blood and urine, or of vitamins, or of the concentration of bile acids, adrenalin, albumin and globulin in the blood (Voluyskaya, 1953).

An inhibition of the hematopoietic apparatus is very characteristic of the influenzal infection, which is expressed in leukopenia and thrombocytopenia (Dubnyakova, 1954). These changes are so pronounced that they can serve as one of the signs for differentiating virus influenza from acute catarrhs of the respiratory tract. Inhibition of the hematopoietic system has been studied in detail by Korovin (1937, 1939) and Kolyaditskaya (1948), who regularly found leukopenia, neutropenia, eosinophilia and a distinct toxic granulation in the neutrophils -- indices of a severe toxic trauma to leukopoiesis -- in influenza.

A distinct suppression of the protective functions of the body is observed in influenza, which is expressed particularly in a suppression of phagocytosis. This problem has been studied in particular detail by Zakstel'skaya and Gudkova as well as by Kornyushenko.

The inhibition of phagocytosis in patients with influenza has been distinctly shown in the investigations of Zakstel'skaya (1952, 1954). In Table 58 the opsono-phagocytic indices are presented in healthy people with respect to the staphylococcus, the most constant inhabitant of the nasopharynx and the most common pathogen of complications in influenza. The percentage of leucocytes phagocytizing staphylococci ranged from 68 to 100, and the opsono-phagocytic index ranged from 8.8 to 17.

A completely different picture has been observed in patients with influenza (Table 59). In the acute stage of the disease (patients Nos 13-44) the percentage of leucocytes phagocytizing the staphylococci was reduced to 12-64, while the opsono-phagocytic index reached very low figures (0.6-1.4). In a later stage of the disease and during the period of convalescence the phagocytic indices again returned to normal (patients Nos 1-12).

Table 58

Phagocytosis of Staphylococci by Leucocytes of Healthy People

Index	Number of People Investigated							
	1	2	3	4	5	6	7	8
Percentage of phagocytizing leucocytes	94	72	100	70	82	80	71	90
Opsono-phagocytic index	17	12	16	10,5	14,1	12,8	10,3	12
Presence of non-phagocytized microbes	+	+	+	+	+	+	+	+

On examination of a control group of patients with acute catarrhs in whom the diagnosis of influenza was made incorrectly the percentage of phagocytosis and opsono-phagocytic indices were nearer to the indices found in healthy people although they were reduced (respectively, 37-88 percent and 4-10,5).

Gudkova (1952, 1953) showed that in influenza a considerable suppression of the phagocytic reactions in the tonsils is observed. By using the method of investigating the phagocytic reaction in impressions taken from the tonsils, she found that a pronounced suppression of the phagocytic reactions in the tonsillar tissues is observed in parallel with the reduction in phagocytic activity of the blood leucocytes during the first three or four days of influenza, which did not occur in the acute catarrhs of the respiratory tract. The method which she developed gave entirely consistent results when compared with serological investigations, on the basis of which it was recommended for the diagnosis of influenza. In Fig. 147 typical curves are shown of the phagocytic reactions in influenza patients. For the purpose of comparison it is fitting to compare them with phagocytic indices in the acute catarrhs (Fig. 148). The comparison makes it possible to show distinctly the pronounced suppression of the phagocytic reactions in influenza patients during the first three or four days of the disease.

Table 59

Phagocytosis of Staphylococci by Blood Leucocytes of Patients With Virus Influenza

Index	Number of people investigated							
	1	2	3	4	5	6	11	12
Percentage of phagocytizing leucocytes	62	93	51	60	16	68	82	72
Opsono–phagocytic index	4	5,5	2	2,4	0,6	8	0,9	1,4
Day of taking blood	7 th	6 th	7 th	6 th	3-rd	8-th	5 th	3- rd
Increase in antibodies[1]	A_1 8	A_1 -4	B_1 2	B_1 4	A_1–4	A_1 2	A_1 -8	A_1–2
Complications	+	+	+	+	+	+	+	+

Index	Number of people investigated							
	13	14	19	20	33	41	42	44
Percentage of phagocytizing leucocytes	14	12	18	29	30	64	52	18
Opsono–phagocytic index	0,6	0,5	1	1,3	2	0,6	2,5	1,44
Day of taking blood	2 nd	2 nd	2 nd	2 nd	3 rd	2 nd	2 nd	2 nd
Increase in antibodies [1]	A_1- 4	A_1–2	A_1 4	A_1–4	A_1–8	A_1–4	A_1–2	A_1–4
Complications	+	+	+	—	—	—	—	—

[1] The figures represent the coefficients of increase in the titer; the letters, the type of virus.

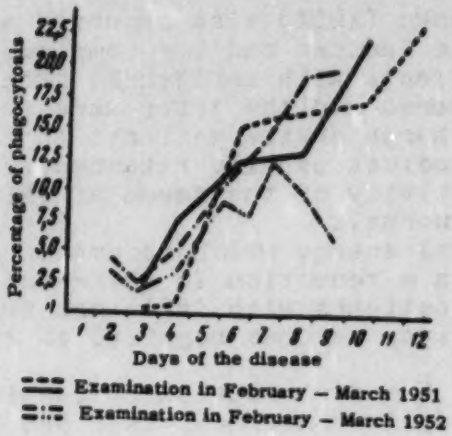

Fig. 147. Average Indices of Phagocytic Reaction in Patients With Virus Influenza.

Broken lines -- phagocytosis in impressions from tonsils; solid lines -- phagocytosis in peripheral blood (Gudkova, 1953).

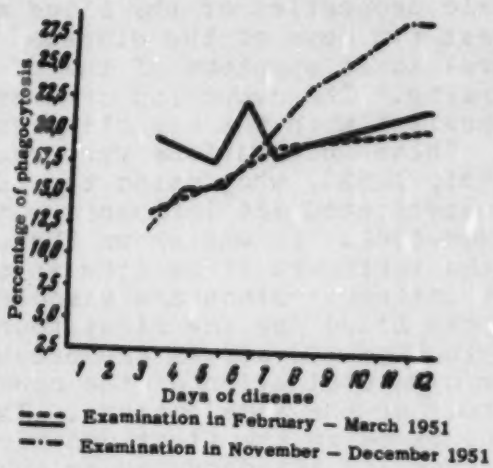

Fig. 148. Average Indices of Phagocytic Reaction in Patients With Acute Catarrh of the Upper Respiratory Tract (Gudkova, 1953).

Kornyushenko (1952) also observed a marked reduction in the phagocytic indices and the complementary activity of the serum in patients with influenza. The more severe course the influenza had the lower were these indices. By the time of discharge of the patients on the 10-12th day the phagocytic indices usually returned to normal, but the complementary activity of the serum at this time had usually not returned to normal.

The general anergy which occurs in influenza is expressed also in a reduction in the specific immunobiological indices: in patients with influenza the Pirquet reaction, previously positive, becomes negative in 72 percent (Debré, 1918).

As far as the distribution of the influenza virus in the patient's body is concerned, this problem has been little studied. Until recently the virus could be isolated only from respiratory organs, in which its multiplication occurs. However, the characteristics of the pathogenesis of influenza indicate that the virus enters the blood stream, and many symptoms of intoxication are apparently associated with this. Recently, it has been established through the direct observations of Solov'yev, Zakstel'skaya and Tarusov (1950) on patients with influenza that in the acute stage of the disease the blood possesses pronounced toxic properties. The toxic properties of the blood are most pronounced during the first two days of the disease, that is, at a time when the general toxic symptoms of the disease reach their greatest intensity. The reduction of these manifestations proceeds in parallel with the reduction in toxic properties of the blood. These observations were continued by Zakstel'skaya (1951, 1953), who, using the constriction test of Tarusov, investigated 124 influenza patients during an outbreak in 1948-1949. It was shown through these investigations that the influenza virus (the specific toxic properties of the patients' blood are associated with it) circulates in the blood for the first four or five days of the disease. In Fig. 149 curves are presented obtained from the dynamic investigation of the constrictive properties of the blood of the same patient. The pronounced toxicity of the blood on the first and partly on the second day of the disease and its decrease on subsequent days are distinctly seen.

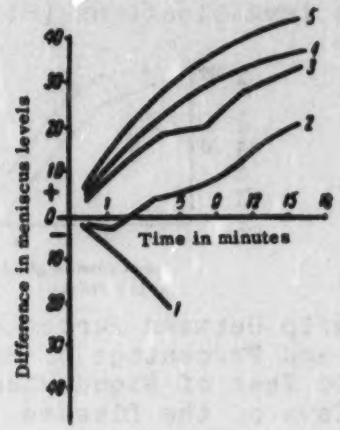

Fig. 149. Change in Toxicity of the Blood in Patient B Depending on Day of the Disease.

Curves 2-5 show the course of the constriction reactions of mouse tissues with the blood of patient B; curve 1, the normal tissue constriction of the mouse, according to days of the disease (Zakstel'skaya, 1953).

In Table 60 data are presented of the repeated examinations of patients' blood for the concentration of the toxic factor of the influenza virus.

Table 60

Data of Repeated Examination of Patients' Blood

Patient	Toxicity of blood according to days of the disease					Temperature of the body according to the days of the disease				
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
A.	+++	+++	+	+		39°	39,3°	38,8°	37,7°	36,1°
B.	+++	+++	−	+		38,9°	37,7°	37°		
Zh.	+++	++	+	+		38,2°	37,8°	37,2°		

The strict correlation between the concentration of the toxic factor (that is, virus) in the blood and the height of the fever attracts attention. This correlation is distinctly expressed also in the comparison in the re-

sults of large-scale investigations (Fig. 150).

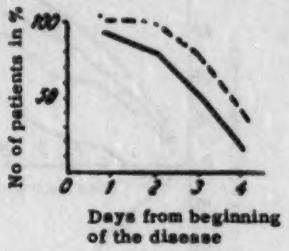

Fig. 150. Relationship Between Percentage of Febrile Patients and Percentage of Patients With Positive Toxic Test of Blood When Examined on Various Days of the Disease.

---- percentage of febrile patients; -.-. percentage of patients with positive toxic blood tests (Zakstel'skaya, 1953).

Data concerning its isolation in the urine also constitute evidence of the circulation of the influenza virus in the blood of the patients.

Zakstel'skaya (1953), using a sensitive method, showed that the influenza virus can be found in the urine of patients. The influenza virus strain (VO) which she isolated from the urine of children proved to be a typical A-prime virus. These data indicate the penetration of the virus beyond the limits of the lungs during the infectious process.

47. The Role of Secondary Microflora in Influenza

The significance of bacterial infection in influenza in people was studied in parallel with the experimental data presented above.

Drobyshevskaya, Ostrovskaya and Shishkina (1937) made careful investigations of the microflora of the respiratory tract in patients with influenza dynamically according to periods: the first six days of the disease, the second six days of the disease, and the period between the 13th and the 40th day (convalescence). The Pfeiffer bacillus was isolated during the first period in 90 percent of the patients; in the second period, in 58 percent; in the third period, in 34 percent, whereby in 42 percent of the cases virulent forms were isolated. The corresponding findings of the pneumococcus (the majority were type IV) amounted to 100-76-56 percent; of the Micrococcus catarrhalis, 84-50-31 percent; of the hemolytic streptococcus, 28-17-12

percent. The quantitative characteristics of the microflora isolated attract attention: the number of microbes plated out during the acute period exceeded their number during convalescence by 1,000 times.

Smorodintsev, Gulamov and Drobyshevskiy (1948)studied the dynamics of secondary microflora in influenza during epidemic outbreaks in 1936 (A virus) and 1940 (B virus). The authors characterized the changes in the microflora of the upper respiratory tract in influenza as an intense mobilization of a broad series of conditionally pathogenic microbes during the first few days of the disease. The cessation of the clinical signs was accompanied by a considerable quantitative reduction or complete disappearance of the bacteria in the majority of patients. The isolation rate of the various microbes is characterized by the following data (Table 60a).

Table 60a

Isolation Rate of Bacteria Dynamically in A and B Influenza in Percentages of the Number of Persons Investigated According to Six-Day Periods

Name of microbe	Influenza A			Influenza B		
	1st	2nd	3rd & later	1st	2nd	3rd & later
Pfeiffer bacillus	90	58	34	63	43	26
Pneumococcus	100	76	56	79	73	32
Micrococcus catarrhalis	84	50	31	--	—	—
Streptococcus hemolyticus	28	17	12	65	—	—

The concentration of microbes in the upper respiratory tract changed markedly. Thus, during the first six-day period the average number of Pfeiffer bacilli for the entire washing was equal to more than 10,000 microbial cells; of pneumococci, about 100,000. At the beginning of the second six-day period, their number decreased by 100-1,000 times, and after the 13th day only solitary cells were encountered throughout the material. Similar data were presented in the work of Subbotina (1954).

Glukhov (1949), on investigating influenza outbreaks in Leningrad in 1945 and 1946, found a streptococcal antigen in 70.2 percent of the patients in the serum (during the period of convalescence). The complications were produced in large part by the streptococcus.

According to the data of Krivaya-Usherenko (1952), in influenza patients staphylococci, streptococci, pneumococci and the Bacilli influenzae are found most frequently in the throat; in addition, the Micrococci catarrhalis, diplobacteria of Friedländer and other microbes are found. No significant differences were observed in the species composition of these microbes in patients with influenza and acute catarrhs. None of the microbes was isolated from all of the patients; however, the staphylococci were most frequent and abundant. In complicated influenza the number of microbes increased markedly, whereas in uncomplicated influenza the increase in their number was slight and was not maintained long.

In the work of Zhdanov and co-authors (1953), who made a comparative study of the bacterial microflora in influenza and acute catarrhs, data were obtained which were similar to the results of Smorodintsev's and his co-workers' research. The authors did not find any essential differences in the composition or quantitative relationships of bacteria isolated in patients with influenza and acute catarrhs. The serological investigations -- the agglutination reaction of bacteria isolated from the patients with their sera taken during the acute period and during the period of convalescence -- showed a variegated picture. It was impossible to demonstrate any serological changes in the patients with respect to any of the most frequently isolated microbes (staphylococci, streptococci). Here, it is fitting to analyze the influence of cold stimuli on the development of the influenzal infection in men. Existing observations are of an empirical nature and indicate, in general, that the chilling factor may aggravate the course of influenza. More exact research pertains to the effect of the cold factor on the development of infection produced by conditionally pathogenic microflora of the respiratory tract. We consider it possible to dwell briefly on these data, because secondary infection is of importance in the pathogenesis of influenza.

The nature of the effect of the cold factor has been demonstrated by the investigations of Marshak, Koyranskiy, and Epshteyn. Marshak and co-authors (1935, 1938), in examining volunteers, established the fact that chilling of localized areas of the skin produces both a local reflex reaction expressed in a constriction of the blood vessels and a change in the blood supply and corresponding vascular reactions and an increase in the secretion of the mucous membranes of the upper respiratory tract.

According to the data of Koyranskiy (1940, 1948), the reflex reaction to local chilling involves a number of organs and systems. Here, not uncommonly, weak cold stimuli,

particularly a replacement of one temperature routine by another, lead to the development of the most pronounced reactions.

Epshteyn and co-workers (1948) made a detailed study of the effect of local chilling on the development of infection in the nasopharynx in volunteers, infection produced by conditionally pathogenic microflora. The introduction into the respiratory tract per se of microflora isolated from the nasopharynx did not produce any kind of pathological reactions. If the persons examined were exposed to local chilling they developed catarrhal signs in the upper respiratory tract, chiefly mild. The possibility of "toughening" people to repeated episodes of chilling was shown, and the latter, in this case, were not accompanied by any pathological signs.

Wildführ (1950) used a cold stimulus (inhalation of cold air into the mouth and chilling of the feet) on normal persons and those predisposed to colds. In the latter, the alexin and opsonin content in the blood decreased sharply; in normal people a slight decrease was observed.

48. Critical Evaluation of Certain Views on the Pathogenesis of Influenza

As a conclusion to this chapter it seems expedient to give a critical evaluation of certain views on the pathogenesis of influenza and to make the necessary generalizations.

Even in the old works of the Russian clinicians influenza was recorded as a general disease wherein general intoxication of the body was most important, along with the local inflammatory processes. The main result of the local effect of the influenza pathogen is a necrosis of the epithelium and an inflammation of the mucous membrane of the respiratory tract in mild cases and a necrotic hemorrhagic pneumonia in severe cases. The general toxic effect of the influenza pathogen consists mainly of the involvement of the nervous system, cardiovascular system, and a suppression of the protective forces of the body. If we do not dwell on the unimportant details, these are the kind of statements which are found in the works of Voskresenskiy (1827), Filatov (1883), Glinchikov (1922), Konchalovskiy (1927), Ivashentsov (1932), Rozenberg (1934) and other clinicians who studied influenza. As may be seen from everything which has been presented in this chapter such a manner of consideration of the pathogenesis of influenza is undoubtedly correct, although it is of a somewhat general character, which was associated with the level of our knowledge concerning influenza at the time of discovery of its pathogens.

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After the discovery of the influenza pathogens a tendency was outlined in the foreign literature toward considering the pathogenesis of influenza a purely local process. Views of this kind were expressed by Francis (1935), Andrewes (1942), Stuart-Harris (1947), Burnet (1946). This idea was expressed most clearly by Burnet, according to which the nature of the pathogenesis of influenza amounts to an invasion of the respiratory tract cells by the virus and intracellular multiplication which produces necrosis of the cells. The main factor determining the acuteness and seriousness of the initial signs of influenza is specifically the rapidity of spread of the infection along the epithelium of the respiratory tract. Typical "toxic" signs at the beginning of the disease, in his opinion, should be considered the result of absorption of products of diffuse and almost simultaneous necrosis of a large number of epithelial cells. Therefore, according to Burnet, the entire situation lies in the necrosis of epithelium lining the respiratory tract and in the quantity of decomposition products which enter the body from there. With less necrosis less decomposition products are absorbed, and the toxic state is less pronounced.

It seems to us that this point of view is erroneous and simplifies the complicated processes which play a part in the development of the influenzal infection. Burnet does not take into consideration either the intoxication which develops in connection with the absorption of the influenza virus (specifically of the virus and not of the decomposition products of the cells) nor of the suppression of the protective forces of the body, as a result of which bacterial infection develops in influenza as the second stage; finally, he does not take into consideration the effect of the virus on the nervous system, the direct consequence of which is the occurrence of cardiovascular disorders characteristic of this infection.

Another incorrect tendency in the interpretation of the pathogenesis of influenza was delineated in the Soviet literature in connection with the study of the role of the nervous system in infection and immunity. This tendency was most distinctly expressed in the works of Sakharov (1953), Stepanov and Zaytseva (1953). In Sakharov's opinion, the infectious process in influenza begins with a primary stimulation of the mucosal receptors and a subsequent development of the pathological process in the upper respiratory tract in contrast to acute catarrhs, in which the upper respiratory is involved secondarily in the disease process, along the type of reflected reflex reactions. Stepanov and Zaytseva believe that influenza is a neurodystrophic process the initial stage of which is an

effect of the virus on the physiological mechanisms (nerve receptors) in the respiratory tract; afterwards, changes occur in the functional state of the nervous system in the form of a disturbance in the conditioned reflex activity of the body, various organs and systems.

It seems to us that this point of view is just as erroneous as the first one, because the complex and rapid processes which play a part in influenza have been included under the universal scholastic concept of a neurodystrophic process; unimportant conditioned-reflex changes observed in influenza are made most prominent. The pathogenesis of any infection can be given such a formulation, whereas influenzal infection, like any other, has its own specific features characteristic of it alone. Apparently, the simplified interpretation of the idea of nervism and of the physiological teaching of I. P. Pavlov is a result of this incorrect interpretation of the pathogenesis of influenza.

Therefore, we can agree with the opinion of Ado (1935), who warns against a primitive interpretation of the idea of nervism in its application to the pathogenesis of influenza. He emphasizes that influenza viruses in experiments with the experimental infection in mice and rats constitute distinct nervous system stimuli which disturb its reflex activity. The point of their application is primarily the central end of the cortical analyzer, but can also be other links in the reflex arc.

The study of the pathogenesis of virus infections, however, does not give us any grounds for accepting the reflex nature of the damage produced by the viruses in the sick organism. The reflex activity in the nervous system is disturbed as a result of the injury of various links of reflex arcs by the virus.

It seems expedient to us to emphasize the following most important characteristics of the pathogenesis of influenza. The influenza virus, coming into contact with the mucous membranes of the respiratory tract, enters into an enzymic reaction with the mucoid substances of the respiratory secretion and of the membranes of the epithelial cells. Only after contact with the latter, as a result of which the integrity of the cell membranes is impaired, are the penetration of the virus into the cell and its multiplication possible. The multiplication process occurs cyclically, whereas with the exit of the virus from the cell a destruction of the cells and of the mucoid-rich intercellular substance occurs. Necrosis of the cells and a destruction of the interstitial substance leads to the development of a reactive inflammatory process, which, however, does not prevent the further multiplication of the virus at least in the first stages of the infection. The

multiplying virus suppresses the activity of the leucocytes and other cells of the reticulo-endothelial system, increases the permeability of the tissue, as a result of which resorption of the virus occurs, and it enters the blood stream. After being carried throughout the body by the blood, the virus multiplies in other organs and tissues, manifesting its toxic effect, which is expressed particularly in the nervous system. A disturbance of the activity of the nervous system (primarily, of the vegetative nervous system) involves the development of cardiovascular disorders, and, to a lesser degree, a disturbance in the activity of other organs and systems. Another result of the multiplication of the influenza virus in the lungs is the penetration of conditionally pathogenic microflora which inhabit the respiratory tract into the tissue. The latter is facilitated by the fact that the integrity of the mucous membrane is impaired (epithelial necrosis), the tissue permeability is increased (destruction of the mucoids of the interstitial substance), and the protective forces of weakened (suppression of the phagocytic activity of the leucocytes). If the process develops further, the virus, which comes repeatedly from the cells, by being adsorbed onto the walls of the pulmonary capillaries destroys them, and a hemorrhagic pneumonia develops. The mass entry of the virus into the blood aggravates the nervous system involvement and brings about an increase in the vascular disorders, and afterwards a destruction of the capillaries occurs which is produced directly by the virus not only in the lungs but also in other organs. In these cases, death occurs either from hemorrhagic pneumonia or from a disturbance in the activity of vital organs, or from cerebral involvement; in the long drawn-out cases, from the development of secondary infection. However, such outcomes are comparatively rare. Usually, the body copes with the infection, mobilizing all its protective forces, as a result of which multiplication of the virus is stopped, and then its death occurs. The virus, after entering the blood, is excreted in the urine by the kidneys; the bacterial microflora which penetrates into the tissue is destroyed, and reparative processes develop, and a special immunity is produced. The nervous system plays an important part specifically in these compensation processes for inflicted injuries and in the production of a specific immunity. However, this problem goes beyond the realm of this chapter and will be analyzed in the chapter on immunity.

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FIFTH CHAPTER

IMMUNITY

49. General Characterization

The development of an influenzal infection produces a reorganization of the reactivity of the entire organism, as a result of which a specific immunity is produced to influenza.

The immunity develops early, beginning with the end of the first week, and after two or three weeks it reaches its greatest strength. It is maintained in such a state for several months, after which it begins a gradual decrease which terminates in a loss of resistance to influenza in the few years after having had the disease. The immunity to influenza is type-specific, associated with the antigenic structure of the pathogens. The A, B, C, and D viruses do not produce any cross immunity to one another; the A and A-prime viruses produce only a partial, weak cross immunity; the varieties of the same virus produce an incomplete immunity to one another. Resistance to influenza is not produced as a result of some kind of single immunity factor but rather is the result of the effect of many factors: antibodies, altered reactivity of the tissues, phagocytosis as well as physiological protective mechanisms (activity of the kidneys, liver, etc.). Here, immunity to influenza is not the simple sum of these factors but rather represents an interrelated complex of them controlled by the activity of the central nervous system.

Below, we shall analyze the various immunity factors in influenza and its characteristics as a whole.

50. Humoral Immunity (Antibodies) in Experimental Influenza

The significance of the humoral factor (antibodies) in the development of active immunity to influenza has been studied in experiments on animals and in observations on people.

Experimental influenzal infection in animals susceptible to influenza is always accompanied by the formation

of antibodies, in parallel with which the resistance of the animals increases to a second infection with the influenza virus (Andrewes, 1939). The antibodies appear in the blood serum in ferrets and mice on the seventh day of experimental infection (Francis, 1935), reach a maximum after two or three weeks, are maintained for a certain time at this level, after which their concentration gradually falls.

Smorodintsev and Shishkina (1940) studied the part of the humoral factor in the mechanism of active immunity to influenzal infection of mice. The group of mice were immunized with live virus by means of a quadruple injection of it under the skin, by means of a triple injection intraperitoneally and by means of a double inhalation of sublethal doses of the virus. After various intervals of time resistance of the animals was found to intranasal infection with different doses of the virus and simultaneously an antibody titer was found in the blood. In these experiments a distinct correlation was established between the titer of virulent antibodies and the resistance of mice to the influenzal infection. In Fig. 151 certain results of these investigations are presented.

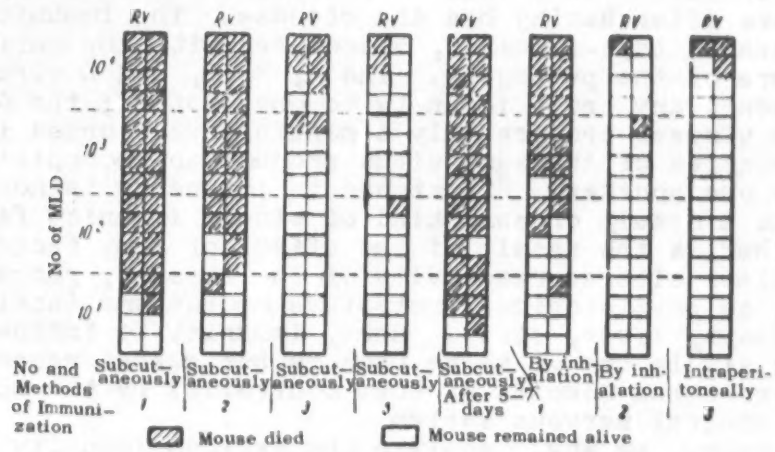

Fig. 151. Humoral Index and Resistance of Mice Immunized With Live Virus.

R -- resistance of mice; V -- viricidal power of the blood of these mice (Smorodintsev and Shishkina, 1940).

From Fig. 151 it is seen that after subcutaneous immunization the increase in the number of immunizations increases simultaneously with the resistance of the mice to the influenzal infection and with the content of viricidal

antibodies in their blood. The decrease in the titer in the blood after five to seven days is accompanied by a corresponding decrease in the resistance to influenza. Very similar relationships are observed after intraperitoneal and intranasal immunization. A close association was shown between the resistance to the virus-neutralizing antibodies in the blood by comparing the results of immunization with live and formalin-killed virus.

In comparing the resistance of the mice immunized actively and passively, however, completely different relationships were found. Despite the fact that the antibody titer in the blood of mice which received influenza antiserum exceeded that in actively immunized animals died from 100 MLD of the virus, whereas the actively immunized animals withstood an infection of 10,000 MLD of the virus.

The relationships changed only when the serum was not injected into the blood but rather into the respiratory tract. In this case even after the injection of small quantities of the serum the resistance of the mice to influenzal infection increased markedly, despite the fact that a negligible quantity of antibodies was circulating in the blood.

In evaluating the results of these investigations, the authors came to the conclusion that humoral factors (specific influenzal viricidal antibodies) play the main part in creating immunity to influenza. Thereby, in connection with the marked pneumotropism of the influenza virus antibodies which penetrate from the blood into the epithelial lining of the respiratory mucosa are of principal importance.

In order to confirm these considerations, the authors (Smorodintsev and Shishkina, 1946) studied the dynamics of the influenza virus in the bodies of mice immunized actively and passively in which the antibodies had been removed from the organs by washing on filter membranes and by total irrigation of the vascular system. In Fig. 152 the dynamics of the virus are shown in the lungs in normal and passively and actively immunized animals when determined without elution and with elution of the antibodies.

Similar data were obtained also in the determination of the virus dynamics in a subcutaneous focus.

From these experiments the authors drew the conclusion that the antibodies, by combining with the virus, do not inactivate it; therefore, after elution of the antibodies from the organs the virus can still be found there in concentrations which are not much different from that in non-immune animals. The authors also come to the conclusion that aside from antibodies there are no additional defensive factors in the bodies of actively immunized animals.

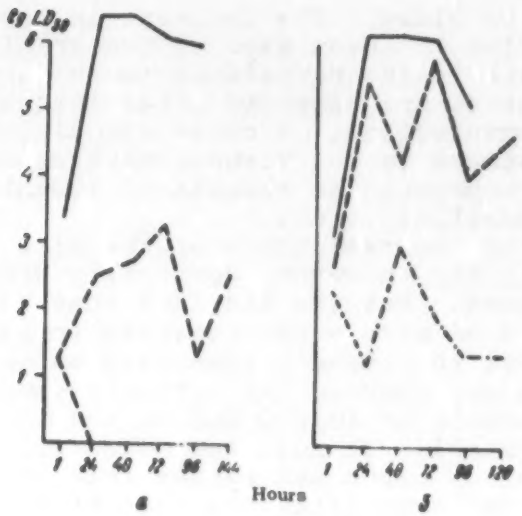

Fig. 152. a, b. Dynamics of the Virus in the Lungs of Normal, Actively and Passively Immunized Mice Following Intranasal Infection With 10^5 MLD of the Influenza Virus and a Determination of It by Various Methods.

____Normal mice; - - - actively immunized mice; -.-.-. passively immunized mice (Smorodintsev and Shishkina, 1946).

Francis (1939) showed, in experiments on ferrets, a relationship between the quantity of virus injected for immunization, the antibody level in the blood and the susceptibility to influenza after subcutaneous immunization. When there is an adequate quantity of the injected virus the animals do not become sick after infection by the respiratory tract. Similar results were obtained by this author after the immunization of mice intraperitoneally.

The protective effect of the sera to the A and B viruses was strictly specific (Zellat and Henle, 1941).

This viewpoint was confirmed and developed in the investigations of Fazekas de St. Groth, Donnely and Graham (1950-1951). In experiments on mice these authors showed that antibodies (antihemagglutinins) appear in the blood and in the bronchial secretion on the seventh-eighth day after infection of the animals with the virus, reach maximum titers on the 12th day, and then the titer gradually falls. The quantity of antibodies in the nasal secretion amounts to five percent of their quantity in the blood. During the course of the experiments the authors directed attention to the facts indicating a lack of correlation between the antibody concentration in the blood and the resistance of the mice after various methods of immunization, which had been mentioned in passing by Smorodintsev

and Shishkina in the works presented above. After intranasal injection of sublethal doses of the live virus the indices of resistance exceed the corresponding indices for intraperitoneal and subcutaneous immunization by 10^5 and 10^6 times. This phenomenon is observed, although to a lesser degree, after immunization with formalinized and heated virus. The authors' data, like those of Smorodintsev, explain this phenomenon by the differences in concentration of antibodies in the bronchial secretion after different methods of immunization.

They point out that a close correlation exists between the resistance of animals to the virus and the antibody titer in the bronchial secretion, and that there is no correlation between the resistance and blood antibodies.

Without presenting the content of these investigations in detail, we should like to note that similar data and conclusions concerning the role of antibodies in the development of immunity are also present in the works of Smith, Andrewes and Laidlaw (1933), Francis and Magill (1935), Francis and Stuart-Harris (1938), Solov'yev (1945), Zakstel'skaya (1953) and other investigators who studied immunity in influenza.

In experiments with infection of monkeys through the respiratory tract, Vieuchange (1939) and Burnet (1941) also found a distinct correlation between the antibody level and the resistance of animals to reinfection.

Antibodies against the influenza virus, as against other virus infections, are associated with the gamma-globulin fraction of the serum (Wyckoff and Rhian, 1945).

According to the data of Solov'yev (1950), antibodies may be formed not only in the intact organism but also in tissue cultures of preliminarily immunized animals. By using the method of cultivation of bronchial tissue and tissue of the lungs of immunized rats and mice in vitro, the author came to the conclusion that the local formation of antibodies was possible, and that these were found in higher titer after intranasal immunization than after pararespiratory injection of the antigen.

The mechanism of reaction of the antibody with the virus was originally considered to be an inactivation of the virus by the antibodies which neutralized it (viricidal) (Laidlaw and others, 1935; Francis and Magill, 1935; Smorodintsev and co-authors, 1936; Zil'ber and co-authors, 1937, and others).

Smorodintsev and Shishkina (1945) soon showed, however, that the influenza virus is not destroyed by the specific antibodies when it is neutralized by them. By using the method of dilution in physiological solution, the method of electrophoresis and adsorption in kaolin, the authors reactivated the influenza virus from neutral mix-

tures, and from mixtures of the virus with hyperimmune serum, on animal charcoal. By means of adsorption onto colloidal Elford filters and supercentrifugation it was possible to obtain a quantitative characterization of the virus which had been liberated from the mixtures with neutralizing antibodies. In this process, the duration of contact and the intensity of contact (temperature, concentration of components) did not reduce the yield of reactivated virus.

The presence of active virus in these experiments was shown by the reproduction of the infection in mice by means of intranasal infection of them.

Reactivation of the virus from its mixtures with antibodies was also observed by Francis and Magill (1935), Burnet, Keogh and Lush (1937), and Taylor (1941), who mention the reversibility of the relationship of the virus with antibodies only in the early stages of their reaction.

Particularly, McKee and Hayle (1946) and Hultin and McKee (1952) showed that the allantoic virus when neutralized or hyperneutralized by specific serum can be reactivated if concentrated killed virus is added to the mixture. The active virus can be passaged in mice and chick embryos in these cases.

For the purpose of understanding the mechanism of reaction of the influenza virus with proteins the investigations of Tovarnitskiy (1945-1950) with respect to virus-protein complexes are of importance. The author showed that the influenza virus combines with the enzyme papain, of protein nature, coming down in the precipitate and thereby losing its biological activity. The complex is of the nature of a salt and can be split into its component elements by means of concentrated salt solutions with restoration of the original virus activity. Similar complexes are formed when the influenza virus combines with protamines, globin, cytochrome C and clupeine, the isoelectric point of which lies at a more alkaline level than that of the influenza virus. These complexes are insoluble in water (they come down in the precipitate). Like antigen-antibody complexes they are biologically inactive or only slightly active, despite the presence of a potentially virulent virus in them. After immunization of mice the virus-clupeine and virus-zephyrol complexes maintain the antigenic properties of the influenza virus (Peterson, 1949).

In evaluating the results of these investigations Tovarnitskiy believes that they open up perspectives for the study of the functional groups of the viruses which play an important part in their reproduction and the determinant groups which determine their antigenic and immunogenic properties.

The virus adsorbed onto erythrocytes can be removed from them with immune serum. The inverse relationships, that is, the liberation of the virus by erythrocytes from its complex with antibodies occurs with much greater difficulty (Dreyzin, 1953).

Pille (1950, 1952) showed that antibodies may be transmitted from the mother to the offspring through the placenta and milk. Experiments were performed on rats, rabbits and guinea pigs. Gravid female animals were immunized with the influenza virus, and the antibodies were determined dynamically in females and in the offspring which were born. It was shown through these experiments that in rats the antibodies are transmitted both through the placenta and through the milk; in rabbits and guinea pigs, chiefly through the placenta and to a much lesser degree through the milk.

Passively transmitted antibodies disappear in the fourth-sixth week of the animals' life.

According to the data of Morozenko (1953), virus-neutralizing antibodies are transmitted from an immunized rat to the young newborn rats not only through the placenta but also through the milk at the time of suckling (Fig. 153). In contrast to this, in guinea pigs the antibodies against the influenza virus are transmitted only through the placenta. The latter is associated with the characteristics of digestion of these animals, because the antibodies are contained in the milk of rats and in the milk of guinea pigs. However, in the latter they do not penetrate through the intestinal wall (Fig. 154), whereas in rats the intestine becomes impermeable to the antibodies only when the animals reach the age of three months.

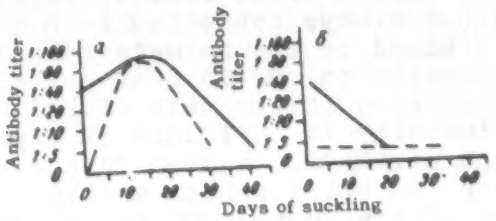

Fig. 153. Virus-Neutralizing Activity in Young Rats Under Conditions of Crossed Natural Feeding With the Milk of Immune (a) and Non-Immune (b) Female Animals.

_____ Young rats born of immune female rats; ---- young rats born of non-immune female rats (Morozenko, 1953).

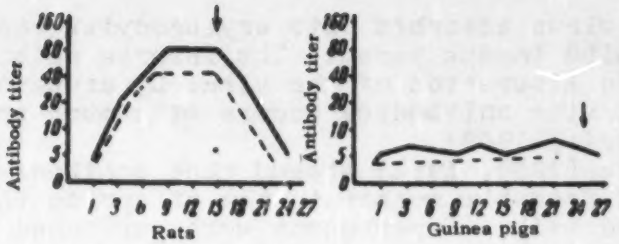

Fig. 154. Change in Antibody Titer in the Blood of Newborn Rats and Guinea Pigs After the Daily Esophageal Instillation of Homologous and Heterologous Immune Sera.

____ Instillation of homologous serum; ---- instillation of heterologous serum; the arrow indicates the time the instillation of the serum is stopped (Morozenko, 1953).

It was established through these experimental investigations, therefore, that specific antibodies are important in the development of immunity to influenza, which gave certain investigators the grounds (Smorodintsev, Fazekas de St. Groth) for considering humoral immunity predominant or even the only type in the development of resistance to influenza.

In the course of time, however, facts began to accumulate which did not confirm the exclusive part of antibodies in the creation of anti-influenzal immunity.

As Francis and Stuart-Harris (1939) showed, ferrets gradually lose their immunity to influenza after having had the infection once. The resistance of various animals to reinfection varies within broad limits; here, the degree of resistance is not always connected with the level of antibodies in the blood of the animals (the authors come to the opposite conclusion, which does not stem from their experiments). According to the data of Fal'kovich and Arkhina (1940), immunity to influenza in experiments on mice occurs before antibodies appear in the blood and disappears while there is still a high antibody titer in the blood, which neutralizes up to 1000 lethal doses of the virus. The authors believe, therefore, that immunity is not so much associated with the presence of antibodies as with the change in the tissue resistance.

Kravchenko and Sekretta (1950, 1953), in a series of investigations which have been presented in the chapter on the pathogenesis of influenza, showed that repeated contact of susceptible cells with small doses of the virus markedly increases the susceptibility to influenzal infection. One of the results of the authors' investigations was the

finding of facts whereby influenza antisera, that is, specific antibodies, not only did not protect the animals against infection but even provoked it. Therefore, specific influenza antisera acted according to the relationship of the reactivity state of the organism to the influenza virus: if it was in a state of normal susceptibility to the virus, the sera exerted a protective effect; if the susceptibility of the organism to the virus was increased, the sera lost their characteristic protective properties.

Tovarnitskiy and Lutikova (1951), using the method of recording mitogenetic radiation, showed that the effect of radiation occurs when the virus is in contact with the tissues of non-immune mice and does not occur with the tissues of actively and passively immunized animals. The removal of antibodies by elution completely prevents the extinction of the mitogenetic radiation in animals immunized passively but does not completely prevent it in animals which have an active immunity. The authors entirely correctly draw the conclusion that other immunity factors exist in influenza besides antibodies.

All these data permit us to draw the conclusion that resistance which develops after influenzal infection in animals is not exhausted by the presence or absence of antibodies in the body even if we consider their distribution in the blood and in the secretion of the respiratory tract tissues.

In the experiments mentioned a study was made chiefly of the virus-neutralizing antibodies, and in later works, of the antihemagglutinins the content of which generally corresponds to the concentration of virus-neutralizing antibodies.

Taking into consideration the important part of toxic properties of influenza viruses in the pathogenesis and immunity to this infection, Zakstel'skaya (1953) studied the significance of antitoxins (antitoxic properties of the sera) in immunity to influenza. In these experiments it was shown that the sera, which possessed neutralizing antibodies, simultaneously possess antitoxic properties. The experiments on rabbits in which the toxic effect of the influenza virus was manifested or was absent depending on the absence or presence of antibodies in the blood and in the anterior chamber of the eye, were particularly demonstrative.

51. Humoral Immunity (Antibodies) in Influenzal Infection in People

Along with the experiments on laboratory animals the significance of humoral immunity in influenza was studied

by means of immunologic investigations of people who had had influenzal infection.

The occurrence of influenza antibodies in people who had had influenza which were specific with respect to the virus producing the disease was established as early as the initial investigations on the isolation of the influenza virus from patients (Smith, Andrewes and Laidlaw, 1933; Francis, 1934; Smorodintsev and others, 1936; Zil'ber, Fal'kovich and Arkhina, 1937, and others). It is such a constant feature that the increase in antibodies in paired sera (taken at the beginning of disease and during the period of convalescence) is considered the most reliable method for diagnosing influenza.

At first, the method of neutralizing the virus with sera in ferrets, mice and chick embryos was used for determining the concentration of antibodies. Soon after, however, it was shown that for the purpose of determining the antibodies these cumbersome and laborious methods of neutralization of the biological activity of the virus could be replaced by the complement-fixation reaction and the hemagglutination-inhibition reaction.

The use of the complement-fixation reaction for determining the antibody titer to influenza was based on the work of Francis and co-authors (1939), Fairbrother and Hoyle (1937), Lush and Burnet (1938), and in the USSR on the experiments of Belikov (1940), whose data were confirmed by numerous subsequent investigations. A comparison of the results of titration of virus-neutralizing antibodies with the results of the complement-fixation reaction showed considerable agreement in the titers of both reactions (Fig. 155), and similar variations in the complement-fixing antibody titers corresponded to variations in the virus-neutralizing antibody titers during influenza epidemics and during the interepidemic period.

The use of the hemagglutination-inhibition reaction for determining the antibody titer to influenza was based on the work of Hirst (1941), McClelland and Hare (1941), and in the USSR, on that of Shubladze and Solov'yev (1953) and was confirmed by numerous verifying investigations. The result of serum titration in the hemagglutination-inhibition reaction agrees to a considerable extent with the result of the determination of virus-neutralizing antibody titers (Fig. 156), and in using this reaction regular variations are observed in the antibody titers in the population during an influenza epidemic and during the interepidemic period.

Finally, the possibility should be mentioned of a study of immunity by means of determining the antibody concentration in the blood which neutralizes the toxic effect

of the influenza virus. Zakstel'skaya (1953) showed that in the blood of convalescents from influenza antitoxins appear which prevent the death of mice and the development of keratitis in rabbits from the toxic effect of the influenza virus. Although these antibodies appear in low titers, a distinct parallelism is observed between them and the antihemagglutinins.

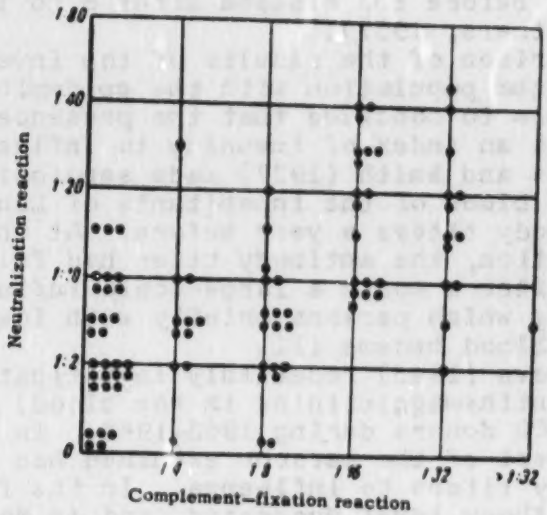

Fig. 155. Correlation Between Virus-Neutralizing Titer and Complement-Fixing Properties of Human Sera (Belikov, 1940).

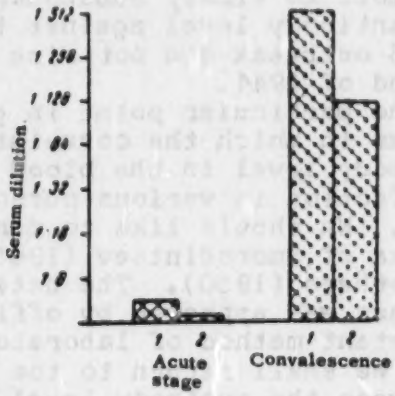

Fig. 156. Hemagglutination-Inhibition Reaction Titers (1) and Neutralization Reaction Titers (2) in Mice of Patient's Influenza Serum in Acute Stage of the Disease and in Convalescence.

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The antitoxins possess a quite marked specificity, and neutralize the toxic properties; in this case, of the PR8 strain to a greater degree than those of the WS strain.

In influenza in people antibodies appear usually on the seventh day of the disease, reach their maximum titer after two weeks, and remain at this level about a month, after which their titer gradually decreases, reaching the antibody level before the disease after 8 to 12 months (Francis and others, 1937).

A comparison of the results of the investigation of antibodies in the population with the epidemiological data made it possible to conclude that the presence of antibodies in the blood is an index of immunity to influenza.

Andrewes and Smith (1937) made serological investigations of the blood of the inhabitants of London who had had high antibody titers a year before. At the time of the second examination, the antibody titer had fallen off sharply, and after a month a large-scale influenza outbreak occurred during which persons chiefly with low antibody titers in the blood became ill.

Vasil'yeva (1945) repeatedly investigated the antibody titers (antihemagglutinins in the blood) against A and B viruses in 500 donors during 1943-1944. In May 1943 a considerable part of the persons examined had high or medium antibody titers to influenza. In the following months the antibody level decreased, and in November 1943 it reached very low figures, particularly to influenza A. In December 1943, a large-scale outbreak of influenza A occurred, after which the average antibody titer to the A virus rose by almost 20 times, subsequently decreasing gradually. The antibody level against the B virus during the December 1943 outbreak did not rise and continued to drop until the end of 1944.

There is no particular point in giving a long list of published works in which the correlation is established between the antibody level in the blood and the resistance to influenzal infection in various persons and in the population generally. We should like to mention only the generalizing works of Smorodintsev (1945), Solov'yev (1945), and Zhdanov and others (1950). The determination of antibodies in influenza was approved by official instructions as the most important method of laboratory diagnosis of this disease (1943). We shall return to the problem of the relationship between the antibody level and resistance to influenza in the chapter on influenza epidemiology. Here, we should like to present only certain data concerning the structure of immunity of the population to influenza, based on a study of the antibody concentration in the blood.

In large-scale quantitative examinations of the sera

of the population for the antibody concentration against the influenza virus, Rickard and co-authors (1941) found that about three percent of the sera neutralize 10^6 LD_{50} of the virus for mice; about 20 percent, from 10^5 to 10^6; over 55 percent, from 10^4 to 10^5; over 75 percent, from 10^3 to 10^4; and only about 20 percent of the sera neutralizes 10^2 or less of the LD_{50} of the virus.

The age variations of the antibodies were studied in detail by Raettig (1947-1948) after the examination of 2230 sera of healthy people. In many children during the first few months of life the antibody titer to influenza was high, falling almost to zero by the age of eight months. In the older age groups it rose constantly, reaching average figures at the age of 10-15 years. After the age of 40 the titer gradually fell.

No great difficulties were noted in the average antibody concentration at different times of the year.

Attempts to tie in the severity of the clinical course of influenza with the nature of the immunobiological reactions were made by Kornyushenko (1953). In the beginning, the antibody titers in patients with different degrees of severity of the course of influenza did not differ from one another notably, but the increase in antibodies in patients severely ill with influenza was more pronounced than when the disease had a mild course.

The more severe course the disease had the greater the complementary activity of the sera was depressed. The formation of antibodies occurred more actively in persons who had not been sick with influenza in the previous two years than in those who had had it in the past.

According to the data of Morozenko (1953), in young children sick with influenza the antibodies are produced in a much lesser concentration than in adults. For the purpose of illustrating this phenomenon we should like to present data obtained by the author in material from an influenza outbreak in 1950 (Table 61).

According to the data of Solov'yev, Ritova and Dudkina (1950), however, young children produce antibodies against the influenzal virus quite actively. In Table 62, data of the examination of children under the age of 1 1/2 months sick with influenza are presented. At the time of the first examination (immediately after birth) they did not have any antibodies in the blood (antihemagglutinins). After having the disease the antibody titer rose to 1:80-1:160, that is, the same thing which usually occurs in adults.

Ritova (1950) showed that the bodies of two-month-old children already produce a good immunity against influenza, which is expressed in the appearance of blood anti-

bodies in high titers as well as antibodies in the nasal secretion after having had the infection.

Table 61

Intensity of Antibody Increase in Connection With the Age of the Patients

Age	No of patients	Change of titer in the second serum					Average index of antibody increase
		No change or doubled	Quadrupled	8-fold increase	16-fold increase	32-fold increase	
1/3—1 year	36	33	3	—	—	—	1,3
1—2 years	33	30	3	—	—	—	1,4
2—3 »	21	18	3	—	—	—	1,6
total of patients	90 (100%)	81 (90%)	9 (10%)	—	—	—	1,4
3—6 years	21	15	3	—	—	—	2,7
6—12 »	19	13	3	3	—	—	2,9
total of patients	40 (100%)	28 (70%)	6 (15%)	6 (15%)	—	—	2,8
0—36 years	53 (100%)	20 (37,7%)	9 (17%)	10 (18,9%)	10 (18,9%)	4 (7,5%)	8,8

Table 62

Immunological Reactions in Children With Influenza

Sick children	Age of child on day of disease	Signs of disease	Highest Temperature	Virus Type	Antibody titer during convalescence
Zhenya T.	37 Days	rhinopharyngitis	39°	B	1:160
Vova B.,	36 »	same	38,5°	B	1:80
Roza Sh.,	17 »	» »	38,5°	B	1:160
Olya F.	21 Days	» »	38,5°	B	1:160
Natasha Zh.	45 days	» »	38,5°	B	1:80
Tonya G.	30 »	rhinitis	normal	B	1:80

Ritova (1953) continued these observations and showed that after having had influenza the antibodies

against the virus which caused the disease accumulate in the tissues of young children in high titers, just as in adults, and are preserved in a stable manner for four months. After seven months, the antibody titers in the blood decrease, and by ten months they are lost.

In Table 63 typical data are presented which were obtained through the examination of children in groups in which influenza outbreaks were observed.

Table 63

Isolation of Influenza Virus Strains From Sick Children and the Antibody Concentration in the Blood

(See next page.)

It is characteristic that the reactions were distinct where strains were used which had been isolated during the

Table 63

No.	Surname of child	Age	Date of disease and examination	Hemagglutination with washings		Type of strain	Serum dilution				Neutralization reaction in chick embryos with serum taken	
				A_1	B		acute period		convalescent period		in acute period	in convalescent period
							A_1	B	A_1	B		
1	Ye–m	1 yr. 9 mos.	14/XI	+	—	A_1	1:20	0	1:320	0	$+10^{-5}$	—
2	Krav	9 yrs. 6 »	15/XI	+	—	A_1	1:20	0	1:160	0	$+10^{-6}$	—
3	Bel.	10 » 6 »	15.XI	+	—	A_1	1:20	0	1:40	0	$+10^{-6}$	—
4	O–va	11 » 6 »	15.XI	—	—	A_1	1:20	0	1:80	0	$+10^{-5}$	—
5	Vas.	9 »	23.XI	—	—	A_1	0	0	1:40	0	$+10^{-7}$	—
6	Yur.	1 yr. 5 »	25.XI	+	—	A_1	0	0	1:160	0	$+10^{-6}$	—
7	Lig	2 yrs.	27/XI	+++	—	A_1	0	1:40	1:160	0	$+10^{-5}$	—
8	B–ayer	1 yr. 5 mos.	27/XI	—	—	A_1	1:20	1:20	1:80	1:120	Not examined	Not examined
9	Tim.	11 »	28/XI	++	—	A_1	0	0	1:80	0	Same	Same
10	Gon..	2 yrs.	28/XI	++	—	A_1	0	20	1:160	1:20	» »	» »
11	Min.	2 »	28/XI	++	—	A_1	0	0	1:80	0	» »	» »

[1] No neutralization reaction

[2] Complete neutralization

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given outbreak and were not so well manifested when old laboratory strains were used. These signs were observed particularly distinctly in 1949. Afterwards, it was made clear that in that year an outbreak of A-prime influenza had first been observed in Moscow; therefore, the use of A strains isolated even at the end of 1948 gave inadequately clear-cut results.

In certain groups typical outbreaks of influenza-like diseases were observed, during which the diseases were indistinguishable clinically from influenza but in which no serological changes were observed in the children's sera.

Afterwards, in the study of these outbreaks a staphylococcal origin of the diseases was shown.

In Fig. 157 data are presented on the change in the antihemagglutinin titer in children recovered from influenza during observation of them for ten months.

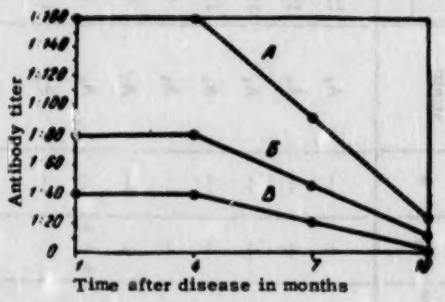

Fig. 157. Dynamics of Actively Acquired Antihemagglutinins in Influenza A.

A -- investigation of ten children; B -- in 18 children; C -- in five children (Ritova, 1953).

The significance of passive immunity to influenza in infancy was studied by Solov'yev, Ritova and Dudkina (1950). The authors investigated the antibody concentration against A and B viruses in newborn children and in their mothers. Thereby, a complete correlation was found between the presence of antibodies in both groups investigated (Table 64).

This correlation pertained both to the virus type and to the approximate antibody titer in the blood, which gave the authors grounds for drawing the conclusion that the latter penetrate into the fetal blood through the placenta in an unobstructed manner.

On investigation of these children two months after birth, the antibody titer remained unchanged in 38 percent; in 37 percent it dropped to zero; and in the others, it dropped to a lesser degree. After seven months, the anti-

body titer fell to zero in almost all the children. It was made clear that during the first two months the children who did not have antibodies in the blood at birth became sick twice as often as children who had antibodies in the blood and in a much more severe form. Therefore, antibodies conferred by the mother protect the children against influenzal infection.

Table 64

Antibodies in Mothers and Their Newborn Children

Subjects	No. of Individuals	No. of children with antibodies against viruses			No antibodies found	Percentage of positive reactions
		A	B	A+B		
Mothers	200	40	42	44	74	63
Newborn children	200	40	42	44	74	63

The investigations were continued by Ritova (1951, 1953), who made a comparative study of the antibody concentration in the sera of mothers and in the children born of them and compared the immunological investigations with epidemiological observations made on the children during the next year.

Two hundred mothers and their newborn children were examined. The results of the examination showed that the mothers and newborn children contain antibodies against influenza in equal quantity. Of the mothers examined antibodies against A and B viruses were found in 63 percent; corresponding results were obtained in the examination of the newborn children. Antibodies were also found in the mother's milk; the type of them coincided with the antibody type in the blood but they were present in small quantity. Corresponding to this, the antibody titer in the blood of newborn children did not change in the next few weeks (Table 65).

Table 65

Dynamics of Concentration of Influenzal Antibodies in Milk and Blood Serum of Mother and Newborn Child

No.	Antibody titer against viruses									
	In the mother				In the newborn child after birth					
	In the milk		In the blood serum		Immediately		After 3 days		After 7 days	
	A	B	A	B	A	B	A	B	A	B
1	1:16	0	1:160	0	1:160	0	1:160	0	1:160	0
2	1:4	0	1:80	0	1:80	0	1:80	0	1:80	0
3	1:8	0	1:80	0	1:80	0	1:80	0	1:80	0
4	1:8	0	1:160	0	1:160	0	1:160	0	1:160	0
5	1:8	0	1:160	0	1:160	0	1:160	0	1:160	0
6	1:8	0	1:140	1:80	1:40	1:80	1:40	1:80	1:40	1:80
7	0	1:32	0	1:80	0	1:80	0	1:80	0	1:80
8	0	1:8	0	1:40	0	1:40	0	1:40	0	1:40
9	1:8	0	1:80	0	1:80	0	1:80	0	1:80	0
10	0	1:8	1:20	1:80	1:20	1:80	1:20	1:80	1:20	1:80
11	1:4	0	1:40	0	1:40	0	1:40	0	1:40	0

In the subsequent observations on the children the antibodies disappeared completely in the second month of life in 37 percent of the children; in 25 percent, the titer did not drop, and in 38 percent it remained unchanged. In this time a study was made of the influenza morbidity rate of children in whom there were antibodies against influenza at the time of birth (first group) and in those in whom there were none (second group). In the first group nine percent of the children became sick; in the second, 38 percent of the children had diseases clinically similar to influenza. On serological examination the diagnosis of influenza was not confirmed in 100 percent of the cases in the first group and in 60 percent of the cases of the second group. On examination after six to seven months the antibodies against influenza were maintained in only five percent of the children, and in low titers (1:20-1:40). A comparison of the dynamics of antibodies against influenza and of the morbidity rate is presented in Fig. 158. In this time, seven children out of 46 in the first group became sick; 11 out of 44 in the second group. In none of those who became sick in the first group was any correlation noted in the type of antibodies previously existent

with the type of antibodies which appeared after the disease.

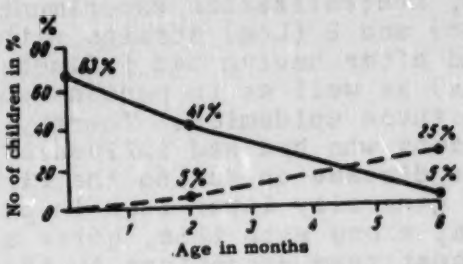

Fig. 158. Dynamics of Passive Immunity and Morbidity Rate in Children Under Six Months of Age.

____ Passive immunity; ---- morbidity rate (Ritova, 1953).

By the age of seven months all the children had lost the antibodies against influenza which they had had. Further observations on both groups showed that the influenza morbidity rate was equal among childfen who had previously had blood antibodies and among children in whom there were no antibodies in the blood.

Similar data were obtained also by Blaskovic (1950) according to whose observations the transfer of antibodies from the mothers to the children occurs both through the placenta and subsequently in the mother's milk. With artificial feeding the antibodies in the blood of the newborn children disappear in the next few months.

Therefore, numerous immunological observations on people attest indisputably to the fact that the existence of a high antibody level in the blood and particularly in the nasal secretion is an index of resistance to influenza inflection and, on the other hand, the absence of them or a low level of them attests to a loss of immunity to influenza.

In recent years, however, data of an opposite nature have accumulated indicating the absence of any correlation between the antibody level in the body and immunity to influenza.

Francis and co-authors (1937) observed cases of influenza in people with high antibody titers in various instances. These authors made their observations in a rural community, reinforcing them with serological examinations. In general, the disease more often affected persons with low titers of the corresponding antibodies in the blood. However, a certain number of persons also became sick who had high indices of neutralizing and complement-fixing antibodies in the blood.

The question of the correlation between the antibody

concentration in the blood and resistance to influenza has been specially studied by Rickard and others (1941). With this aim in view, neutralization experiments were performed on mice of A (PR8) and B (Lee) strains with sera taken from people before and after having had influenza (during the 1939-1941 seasons) as well as in persons who had not had influenza during these epidemics. Thereby, it was clarified that in persons who had had influenza the antibody titers before the disease or during the first few days of the disease were generally lower than in persons who had not had influenza; along with this, quite a few cases were observed which constitued exceptions to this rule.

Similar observations are presented in the work of Salk, Menke and Francis (1945).

This problem has been studied particularly in our laboratory by Zakstel'skaya (1953), Gavrilov (1953) and Lozhkina (1954) with the application of new methods of investigation. The criterion for evaluating the degree of susceptibility to influenza was the degree to which vaccine strains of influenza viruses survived, which had been obtained by means of cultivating them in human tissue cultures. These strains survive on the nasopharyngeal mucosa of people who are not immune to influenza in 100 percent of the cases and are regularly isolated from the nasopharyngeal washings for four to five days. In these observations the absence of any direct connection between the antibody level in the blood and the resistance to influenza was shown. The observations of Gavrilov were particularly demonstrative in this respect; he succeeded in isolating the virus from the nasopharynges of people on the sixth to seventh day after its instillation into persons with high antibody titers in the blood.

In comparing these facts it must be concluded that the blood antibodies are rather an index of a past influenzal infection than an index of the degree of immunity to influenza. This applies also to antibodies in the nasal secretion the level of which, however, can serve in large measure as an index of the specific resistance to influenzal infection. Many investigators who have studied influenza have come to this conclusion. Thus, Smith (1951) points out that although blood antibodies remain for a long time their concentration falls rapidly to a level which does not prevent contact of the virus with the cells through the blood stream.

Without denying the great importance of antibodies in immunity to influenza we consider it necessary to emphasize that antibodies in themselves by far do not determine resistance to influenza and, naturally, are not the only immunity factor in influenza.

52. The Adequacy of Immunological Reactions in Influenza

Still another problem needs to be analyzed -- the specificity and adequacy of serological reactions of the body to the incorporation of the influenza virus.

Large-scale examination of the sera of persons who have had influenza during epidemic outbreaks show that serological changes in general correspond to the antigenic structure of the virus causing the disease. However, it is shown that after more detailed study of this problem that serological reactions of human organisms are not always adequate. In connection with the existence of different variants of the influenza viruses distinguished by a different antigen composition group reactions are found along with the type-specific reactions in performing serological tests with several strains. Thus, the sera of persons who have had influenza caused by the PR8 virus usually show group reactions with the WS virus, while the sera of those who have had A-prime influenza show group reactions with A virus (WS PR8), whereby these reactions usually occur in lower titers than the reactions with the virus of the serological type or variety which caused the disease.

In general, a rise in antibody titer usually occurs in influenza patients which is greater with respect to the homologous strain (with which the given epidemic is associated) than to the old, previously isolated strains. This characteristic feature of immunological reactions of the body in influenza has been noted both in previous and in the latest investigations. The typical relationships of the immunological reactions with respect to the homologous and the old A-prime virus strain are well illustrated in Table 66 taken from the work of Solov'yev, Marennikova and Gutman (1952).

Table 66

Serological Reactions in Influenza Patients During the 1952 Outbreak With A-prime Virus Strains Isolated in 1949 (YeM-prime) and 1952 (S5)

Name of strain	No. of sera containing antibodies	Increase of antibodies by			
		2 times	4 times	8 times	16 or more times
S5	73	18 (24,6%)	18 (24,6%)	19 (26,2%)	18 (24,6%)
YeM'	63	21 (33,2%)	20 (32,3%)	15 (23,4%)	7 (11,1%)

As seen from Table 66, an increase in the antibody titer by eight or more times occurred with the fresh strain in 50.8 percent of the patients and with the previously isolated strain in 34.5 percent of the patients. These relationships were even more pronounced when comparing the intensity of increase in antibodies against the strain isolated from the patient himself with the intensity of the increase in antibodies against the prototype strains. Corresponding data have been presented in Table 67 taken from the work of Zakstel'skaya and Ritova (1954).

Table 67

Increase in Antibodies Against Various Types of Virus in People from Whom Type A-prime Influenza Viruses Were Isolated

Group of persons examined	Surname	Factors of increase in antibodies against viruses			Name of isolated strains
		A	A_1	their own	
Adults	L–v	—	4	8	Liga
»	G–v	—	8	8	6
»	R–a	2	8	8	6
»	F–a	2	4	16	8
»	Ch–a	2	2	16	1
»	M–i	2	4	8	11
»	O–n	2	8	8	13
»	V–y	—	4	8	Vas
»	G–y	2	2	8	Guts
Children born after 1950	Krav–y	—	4	16	Krav
Same	Ch–ov	—	4	8	»
» »	B–v	—	4	8	Bayev
» »	P–v	—	2	4	»
» »	O–a	—	2	4	Ova
» »	Sl–n	—	2	8	»
» »	Gd–v	—	2	8	Gud
» »	St–v	—	2	8	»
» »	S–v	—	2	8	»
» »	G–v	—	4	8	Gar
» »	Gan	—	2	8	Gas
» »	Ba–k	—	2	8	Bak

In Table 67, apart from the more pronounced increase in antibodies against the respective strains, the absence

of group reactions with the sera of children whereas they occur with the sera of adults attracts attention; attention was directed to this in the earlier investigations.

Burnet (1947) in 1937 directed attention to the different nature of immunological reactions in adults and children. He found that in 1937 during an influenza epidemic in Melbourne the sera of the convalescent children neutralized only the local A virus strain (Melbourne), whereas the sera of adults neutralized also the other A virus strains -- WS and swine. Reactions of a similar nature were observed also in performing skin tests in children and in adults with influenza viruses. At that time, Burnet could not explain the causes for these phenomena.

Along with these observations facts gradually began to accumulate attesting to the fact that in certain people the titers of group reactions can be higher than the titers of the specific reactions.

Thus, in 1943, Friedewald (1943) observed cases of disease during which the patients had higher antibody titers against the heterologous strains than against the homologous strains.

Similar facts began to attract attention particularly after the appearance of the A-prime virus. Stuart-Harris and Miller (1947) during an influenza outbreak which was afterwards identified as an outbreak of A-prime influenza, observed higher titers of antibodies against the A virus than against the A-prime virus in a number of patients.

Morgan and others (1948), during the spring outbreak of influenza in Boston in 1947, isolated strains different from the prototype PR8 strain (apparently, these were A-prime virus strains). On serological examinations of influenza patients the antibody level against the strain was higher in many of them than against the strains which had been isolated during the outbreak. Edmunds (1951) investigated 50 paired sera of influenza patients and found that the antibody titers against the PR8 strain were higher than against the local Bar strain isolated during this epidemic. Thus, the average antibody titers in the complement-fixation reaction against the Bar strain was 1.4, and against the PR8 strain 4.3; in the hemagglutination-inhibition reaction these averages were, respectively, 20 and 215.

Particularly striking results have been obtained in our laboratory by Dobrova (1954) in the study of family-apartment house foci of influenza. In some of these foci in which there were multiple cases, which on the basis of epidemiological investigations were indisputably connected with one another and were produced by the same virus in all

the family members who had the disease, an increase in antibodies against different viruses was observed through examination of paired sera of the members of the same family. We should like to present several examples.

In focus A, during the interepidemic period one member of a family became sick, and three days later, two others. In the first, an increase in the antibody titer was noted against the A virus by eight times; a four-fold increase against the A-prime virus, and a four-fold increase against the B virus; in the second, these increases were respectively two, four, and zero times; in the third, by four, eight, and zero times. Therefore, despite the very obvious connection between the diseases the first patient showed serological changes chiefly against the A virus; the other two, against the A-prime virus.

In focus B, during an outbreak of A-prime influenza in a city in 1952, four persons became sick. The intervals between their diseases were equal to two days and one day; there was an indubitable epidemiological connection between them. On serological examination a rise in antibody titer against the A, A-prime and B viruses was observed in the first patient by eight times; in the second, by 16 times against the A virus, by 32 times against the A-prime virus, and by four times against the B virus; in the third, no rise in antibody titer was found, and in the fourth the increase in antibodies was against the A virus by 32 times, and there was a 16-fold increase against the A-prime virus, while there was no rise in antibody titer against the B virus. Therefore, while the etiology was undoubtedly the same, all four patients reacted differently: in one the serological changes were chiefly against the A virus; in another, against the A-prime virus; in a third, against all three viruses; in a fourth, against none of the viruses.

In focus C, during an outbreak of A-prime influenza in 1953 five persons became sick at intervals of one, two and three days. The epidemiological connection of the cases with one another was indubitable. On serological examination no changes in the antibodies were observed in two persons; in the third, there was an increase in the antibody only against the A virus and this was an eight-fold increase; in the fourth, there was an eight-fold increase against the B virus, and a doubling of the titer against the A virus; in the fifth, there was a four-fold increase against the A and B viruses. Therefore, in this case, in the same focus serological changes were observed at times against the A virus and at times against the B virus.

Although we are limiting ourselves to these examples, we should like to point out only that such facts are the

rule rather than the exception.

Thus, during an outbreak of influenza A during the period from 25 January through 1 March 1952, 26 family-apartment house foci were examined in which there were many cases. In eight of these foci the same changes in antibodies were observed in all patients; in six foci the changes did not entirely coincide (some of the patients showed no increase in antibodies); and in 12 foci there were antibody changes against different viruses.

These facts could not help but attract attention, and therefore they were compared with the investigations of Zakstel'skaya (1953), who in our laboratory immunized volunteers with live influenzal vaccine. During the course of these investigations it was made clear that some of them reacted inadequately to immunization with the A-prime virus, producing higher antibody titers against the A virus.

These facts enable us to conclude that infection with the influenzal virus does not always produce an adequate serological reaction of the body, that is, the production of antibodies chiefly against the serological type of virus which caused the disease. This characteristic feature of the immunological reaction of the body cannot be explained as a group reaction with the viruses having a serological similarity to the pathogen causing the disease, because group serological reactions are observed in lower titers than the specific reactions, which is evidenced by many years of practice in work with immune sera obtained from animals.

This phenomenon can be correctly understood only by taking into consideration the characteristics of the influenza epidemiology, the variability of its pathogens, and certain rules and regulations of immunity. Influenza is so widespread among the population that the majority of persons have it several times in their lives in the form of an overt or asymptomatic infection. In the course of time, the antigenic structure of the influenza viruses changes continuously, and therefore, if a person after a few years has the infection a second time caused by the same influenza virus, this virus now has an altered antigenic structure, and at the same time has preserved its antigenic groups in common with the virus which caused the previous disease. Therefore, each new case in the same person is, on the one hand, a repeated immunizing stimulus (with antigenic groups common to both viruses); on the other hand, it represents also an initial immunizing stimulus (with new antigenic groups of the given virus strain). In sum, the antibody spectrum in persons who have had influenza several times is much broader than in persons who have influenza for the first time, because it includes

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antibodies corresponding to the antigenic varieties of the viruses which circulated previously among the population and which had disappeared subsequently. Since the repeated immunizing stimuli produce a rise in antibody titer in greater degree than the initial ones, the antibody increase against the old strains can be much greater than against the new strains if the new strains have preserved their old antigenic groupings. Here, specifically, is the explanation for the facts presented above where in those who had influenza the rise in antibody titer was more pronounced against the old strains than against those producing the disease. Therefore, the highest antibody level in those who are having influenza can be observed against the virus strain with which the given individual first came into contact, and this virus represents the immunologically dominant type (strain) for him. The correctness of these considerations is confirmed by the research of Zakstel'skaya and Ritova conducted in our laboratory.

Zakstel'skaya and Ritova (1954) made serological examinations of influenza patients during 1949-1954, studying 1,796 sera (paired) from 898 patients.

In Table 68 the results are presented of the serological reactions with the sera of patients in whom the increase in antibody titer was observed during the period of convalescence.

Table 68

Relationship Between the Degrees of Different Serological Reactions Against the Influenza Viruses Type A, A-prime, and B in the Sera of Patients Who Have Had Influenza (in percentages)

Year	Total No of cases having antibody increase (100%)	Rise in antibody titer in percent				
		Only against type A	Only against type A'	Mixed reactions in %		
				Increase in type A greater than A'	Increase against type A' greater than against A	Simultaneous increase against A', A and B
March 1949	51	16	11	10	62	1
1949–1950	72	11	17	13	47	12
1951	108	0	35	4	57	4
1952	68	0	46	5	45	4
1953	136	0	74,5	1	24,5	

Note. Blood was taken twice: at the beginning of the disease and after two or three weeks.

During this time outbreaks of A-prime influenza

(1949, 1952 and 1953) and B (1949-1950) influenza occurred in the city, which was confirmed by the isolation of these virus strains. Despite this, in 1949 16 percent of the patients showed a rise in antibody titer against the A virus; in subsequent years mixed reactions were frequently observed; at the same time, the specific importance of reactions against the A virus during these years gradually decreased (Fig. 159).

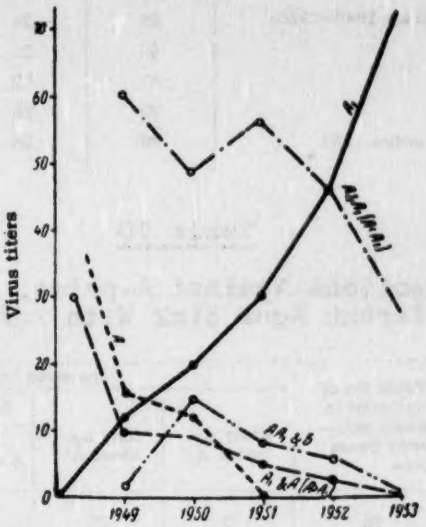

Fig. 159. Interrelationship Between Various Serological Reactions Against Influenza A and A-prime Viruses in Those Who Were Sick During 1949-1953 (Zakstel'skaya and Ritova, 1954).

Table 69 gives us an idea of the average antibody titers against the A and A-prime viruses in patients during various periods.

As is seen from Table 69, the average titers against the A virus in all these years were higher than against the A-prime virus, but the increase in the latter was much more pronounced in later years.

A comparison of the serological reactions in the persons of different age groups is of special interest. This comparison shows a distinctive picture (Table 70).

Table 69

Average Antibody Titers Against A and A-prime Viruses in Those Having Influenza in Different Years

Time of disease \ Virus type	Acute period		Period after recovery	
	A	A_1	A	A_1
March 1949	92	15	186	78
December–January 1949–1950	88	24	126	106
1951	69	26	138	210
1952	61	32	88	201
July 1953	29	26	41	169
November–December 1953	49	28	88	189

Table 70

Serological Reactions Against A-prime, A and B Viruses in Children of Different Ages Sick With Influenza in 1952-1953

Year of birth of the children	Total No of children in whom antibody titers rose	Increase in antibody titer				
		Only against A	Only against A'	Against A and A'		Against A, A' and B
				A and A'	A' and A	
1948	28	0	26	2		—
1949	56	0	34	—	22	—
1950	94	0	64	—	30	—
1951	38	0	12	—	16	—
1952	140	0	140	—		—
1953	102	0	102	—	—	—

In contrast to adults children born after 1948 showed no rise in antibody titers against the A virus only, and in the sera of children born after 1951 the A component was even absent in combination with A-prime, and the latter existed in pure form. The circumstance is particularly distinctly illustrated in Fig. 160.

The average antibody titers against the A and A-prime viruses in these children who were sick in 1953 depended on their ages (Table 71).

Children born in 1948-1949 had had influenza A in the majority of cases before they became sick in 1953; children born in 1952-1953 became sick with influenza for the first time in 1953. Correspondingly, in the first group

of children there was a high antibody titer against the A virus after becoming sick with A-prime influenza (which was the second time they had had influenza), whereas in the second group of children there were pure antibodies against the A-prime viruses alone. It was also characteristic that the A-prime virus disease in 1953 produced quite high antibody titers against the A-prime 1952 virus.

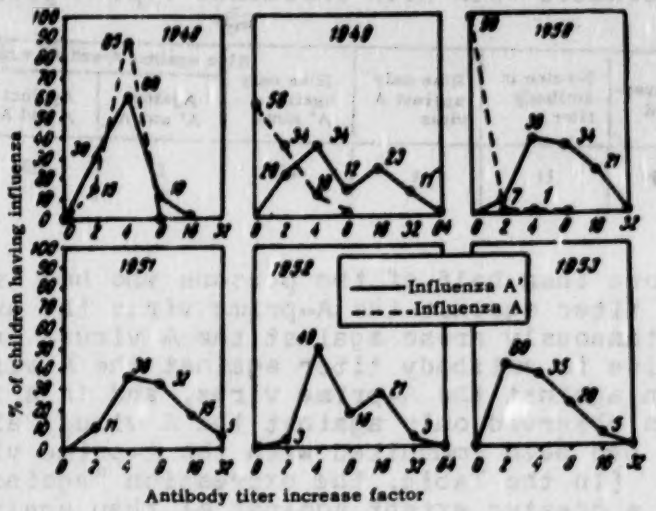

Fig. 160. Type-Specific Reaction Against A and A-prime Viruses in Children Born 1948-1953 Sick With A-prime Influenza (Zakstel'skaya and Ritova, 1954).

Table 71

Average Antibody Titers Against A and A-prime Viruses in Children Sick With A-prime Influenza in 1953

Year of birth	Average antibody titer against virus		
	A	A, 1952	A, 1953
1948	61	38	49
1949	39	43	52
1950	31	41	70
1951	1,3	50	63
1952	0	62	79
1953	0	69	74

Similar data were obtained also in the examination of sera of persons immunized with the A-prime virus (Table 72).

Table 72

Rise in Antibody Titer Against A and A-prime Viruses in Persons Vaccinated With Live Influenza Type A-prime Vaccine

No investigated	No rise in antibody titer	Rise only against A virus	Rise only against A' virus	Rise against A and A' viruses		Rise in antibody titers against A, A' and B viruses
				Against A' and A	Against A and A'	
139	11	4	49	5	68	2

In more than half of the persons who had an increase in antibody titer against the A-prime virus the antibody titer simultaneously arose against the A virus; in certain cases the rise in antibody titer against the A virus was greater than against the A-prime virus, and in a few cases the rise was observed only against the A virus, although the persons had been immunized with the A-prime virus (Table 72). [In the Table, the expression "against A' and A" means to a greater extent against A' than against A; vice versa for A and A'.]

Observations of this kind have been published in recent years also in the foreign literature. Vander Veen (1951) studied the antibody titers in children against the A (PR8) and A-prime (FM1) viruses in Amsterdam. The antibodies against the PR8 virus were encountered in the sera of children under five years of age extremely rarely, whereas they were found against the FM1 virus in 60 percent of those investigated.

Davenport, Hennessy and Francis (1953) studied the antibody spectrum against various strains of influenza viruses in two series of gamma-globulin of which one had been prepared before 1947 and the other, after 1947. The antibody titer against the swine A and B influenza viruses was equally high in both series; the antibody titers against the A-prime viruses were low, whereby in the second series it exceeded the titers in the first series by four times. The sera collected in the interepidemic period 1952, when tested against virus strains isolated from 1936 through 1952, showed different antibody spectra depending on the ages of the persons from whom they were taken. In children antibodies predominated against the A-prime viruses; in adults, against the A and swine influenza viruses; whereby

In children under 10 years there were no antibodies against the A virus, and in persons under 28 years there were no antibodies against the swine influenza virus. The antibodies against the C virus occurred in high titers in all age groups.

Similar work was carried out by Hilleman, Werner and Gauld (1954). Serum investigations of the population in the United States made in 1943-1951 were performed with strains corresponding to the following varieties in the classification of influenza viruses which we and Gorbunova have suggested: A (WS, PR8); A-prime (FM1, FW-1-50), B (Lee, Bon) and C.

In Tables 73 and 74 the average antibody titers are presented against the strains mentioned in the sera investigated.

Table 73

Average Geometric Antibody Titers in Sera Collected in 1951 from Healthy Persons of Different Ages

Age	No of sera	A		A'		B		C	
		WS	PR8	FM1	FW-1-50	Lee	BONV	JJ	1233
2–4 weeks	7	2	13	1	6	9	2	0	
1–2 months	16	0	2	2	9	2	2	6	4
3–5 »	31	2	7	15	41	7	2	27	63
6–8 »	30	2	7	44	64	6	3	24	89
9–11 »	25	2	10	68	52	10	8	10	121
adult	74	32	118	43	46	56	44	33	204

The data presented well illustrate the idea expressed above concerning the characteristics of immunity in influenza. In the latter work the invariably high antibody titer against the C virus attracts attention. If this phenomenon is specific the conclusion should be drawn that this virus is widespread among the population, producing, apparently, asymptomatic forms of infection most often.

In the light of these data, certain results of investigations of previous years become clear. Thus, according to the data of Andrewes, Laidlaw and Smith (1935), in children younger than 12 years it was possible to find antibodies against the A (WS) virus but not against swine influenza. Therefore, this latter virus had stopped circulating among the population at least since 1925.

The material presented in this section substantially supplements and confirms considerations concerning the evolution of influenza viruses presented in the chapter on

variability and, particularly, the idea of the time of occurrence of the swine influenza virus, or more accurately of the original pandemic influenza pathogen type of this virus.

Table 74

Average Geometric Antibody Titers in Sera Obtained 1943 Through 1951 from Adult Healthy Persons

Year	No of sera	A		A_1		B		C	
		WS	PR8	PM1	FW-1-50	Lee	B/NV	No of sera	1233
1943	35	9	18	3	5	10	7	32	263
1944	33	21	78	6	8	1	11	33	182
1945	33	14	65	13	8	24	20	33	129
1946	35	21	96	14	8	71	62	33	191
1947	32	28	91	18	20	65	49	33	159
1948	33	35	96	25	21	47	41	33	162
1949	35	23	83	18	21	59	39	33	219
1950	34	28	60	18	30	38	55	33	200
1951	75	32	118	43	46	56	44	33	204

53. The Role of Phagocytosis in Immunity to Influenza

The significance of phagocytosis and phagocytic activity of the reticulo-endothelial system in influenza have been inadequately studied. The majority of authors who studied this problem believes that phagocytosis does not play any essential part in the development of immunity to influenza.

Smorodintsev and Shishkina (1940) investigated the viricidal activity of leucocytes of mice immune to influenza. In these experiments leucocytes eluted from liquid blood or tissue fluid did not show any viricidal effect when mixed with the influenza virus.

The whole blood of immunized mice did not possess any more pronounced viricidal properties than the serum freed of its formed elements. Very similar data were obtained in the study of the viricidal effect of leucocytes of horses immunized with the influenza virus.

The authors concluded that the leucocytes do not possess any capacity of lowering the activity of the influenza virus, being just as much ballast for the plasma as the erythrocytes, because the excess of formed elements

in the plasma reduced its viricidal activity.

Afterwards, experiments were performed in which the influenza virus was injected intraperitoneally, whereby in part of the animals a local leukocytosis was first produced by the injection of bouillon into the abdominal cavity. In these experiments it was not possible to show any increase in the viricidal effect of leucocytes in the immunized mice either, and the more rapid extinction of the virus in the abdominal cavity of immune mice compared with non-immune mice could be explained by the viricidal effect of the exudate antibodies. After the intravenous injection of the virus into immune mice the virus was distributed approximately uniformly in the organs rich in reticulo-endothelial cells (spleen) and in the organs poor in them (kidneys, brain).

Finally, experiments were performed in which the reticulo-endothelial system was blocked by various methods (iron saccharate, trypan blue), with the removal of the spleen and a combination of these procedures with subsequent infection of the mice. These experiments showed that blockage of the reticulo-endothelial system does not increase the susceptibility of mice to influenzal infection, and a certain increase in susceptibility of animals deprived of a spleen could be explained by the influence of the inflicted trauma. In the same way the production of immunity (resistance to infection, antibody concentration in the blood) was the same in animals in which the reticulo-endothelial system had been first blocked as in the control animals. A certain lag in the production of immunity in splenectomized animals could also be explained by the effect of trauma. The authors confirm the correctness of this explanation by the introduction of a group of laparatomized mice into the experiment, which reacted in the same way as the mice from which the spleen had been removed.

All these experiments enable the authors to conclude that the phagocytic apparatus does not play the essential part in the mechanism of influenzal infection, and that this role undoubtedly belongs to the viricidal antibodies.

Gaydamovich and Solov'yev (1946) studied the part of the lymphatic system in immunity to influenza. In the immunization of rabbits with the influenza virus the antibody concentration in lymph corresponded to its concentration in the blood, but the titers were lower. After extraction of the formed elements and testing of them with influenza virus no antibodies were found in them nor any other viricidal factors.

In a review work, Smorodintsev (1948) concludes that in influenza, as in other virus infections, phagocytic factors do not participate in the fight against the virus; the

leucocytes do not destroy it; humoral factors block the virus but do not destroy it.

Zhumatov (1949), incidentally, showed that if the influenza virus is adsorbed onto chick erythrocytes it is phagocytized by leucocytes in this form. With the destruction of the phagocytes the virus is liberated from them in an active state. If the leucocytes are not destroyed the virus remains in them for the most part, dying before the leucocytes in which they are enclosed.

This gave Smorodintsev (1954) the grounds subsequently for revising somewhat his point of view for considering that phagocytosis is of a certain importance in the development of immunity to influenza. Since a secondary bacterial infection almost always occurs in influenza the influenza virus, being adsorbed onto the bacteria, can be engulfed by phagocytes and perish in them. In this form the virus is phagocytized in cell fragments also by virtue of necrotic processes which develop in the respiratory tract tissues.

In the chapter on pathogenesis it was pointed out that the influenza virus produces leukopenia and depresses phagocytosis. Directing attention to this phenomenon, Sokolov (1953) notes that after infection of mice with the influenza virus a reaction occurs at the site of injection of the virus in the polymorphonuclear leukocytes and in the local macrophage-histiocytes, but they die without exerting any protective effect. In contrast to this, in mice immune to influenza the local leucocytic reaction is not associated with the death of the leucocytes.

In general, the significance of the phagocytic reactions in immunity to influenza has been inadequately studied. Apparently different methodological approaches are needed for a more profound study of these immunity factors in influenza.

54. Significance of Tissue Reactivity in Immunity to Influenza

Investigations made by various authors show indisputably that there is a change in the tissue reactivity of the body and primarily of the respiratory tract after influenzal infection.

A change in the resistance of the epithelium after the infection was shown by Francis and Stuart-Harris (1938) in experiments on ferrets. On the seventh-eighth day after infection, when the bronchi are covered with a multilayered transitional-type epithelium following necrosis of the columnar ciliated epithelium, the former is resistant not only to the influenza virus infection but also to certain traumatizing physico-chemical influences (iontophoresis, zinc sulphate). The columnar ciliated epithelium which

develops later becomes sensitive to the physico-chemical influences mentioned, remaining resistant to reinfection by the influenza virus. At later periods, the resistance of the epithelium to the influenza virus is lost. A parallelism is noted in the concentration of antibodies in the blood and the resistance of the epithelium to the influenza virus.

Pigarevskiy (1953) directed attention to the distinctive mechanism of cellular reactions in influenza which had not been noticed by other investigators. In studying the infectious process in mice infected with influenza virus he found that during the first few hours after the infection a marked increase in the secretion of mucus occurs in the epithelial tissue of the trachea and large bronchi; in the medium-size and small bronchi, where there are no mucus-forming cells, a process of an albuminoid apocrine secretion is found. In the cytoplasm of the epithelium small granules are formed which stain poorly with iron hematoxylin and eosin, which gradually accumulate at the apical pole of the cell and fall off along with it into the bronchial lumen (Fig. 161). After 24-48 hours basophilic and eosinophilic inclusion bodies appear in the epithelial cytoplasm (Fig. 162); the later are extruded through the destroyed apical end of the cell. The cells from which the inclusion bodies have been eliminated and which have lost their apical segments acquire a flattened appearance, being sources for the regeneration of the epithelial layer (Fig. 163).

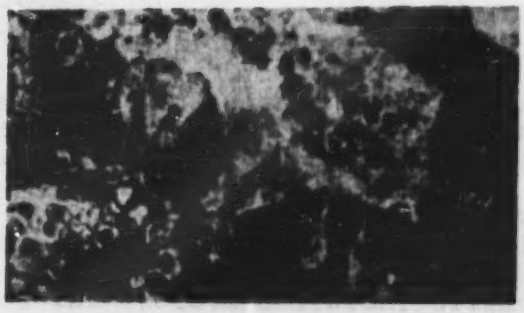

Fig. 161. Markedly Increased Process of Apocrine Secretion in Bronchial Epithelium (Pigarevskiy, 1953).

Fig. 162. Acidophilic (a) and Basophilic (b) Intracellular Inclusion Bodies in the Bronchial Epithelium (Pigarevskiy, 1953).

Fig. 163. Tracheal Epithelium Consisting of a Single Layer of Low, Flattened Cells (Pigarevskiy, 1953).

In evaluating these data the author believes that during the course of development of the infectious process in influenza two groups of protective reactions of the body are observed: an increase in the physiological processes of secretion preventing the penetration of the virus into the epithelial cells and reactive processes in the cells leading to a walling off of the virus (by means of the formation of acidophilic inclusion bodies) and to a subsequent removal of it from the cell with the apical segment, which sloughs off.

The most recent investigations show that tissue resistance after an infection may be associated with the content of mucoids (receptors and inhibitors in the respiratory tracts of mice during an influenzal infection was undertaken in the work of Fazekas St. Groth (1950). After infection of the mice with the influenza virus the number of

receptors remains unchanged for the first four days of the infection. Then their content decreases, reaching a minimum by the seventh day. After this, recovery begins, and by the 11th day their concentration is considerably above normal, returning to it on the 14th day. After large infective doses the first period is shortened.

The content of inhibitors is decreased to zero as early as the first to second day after the infection, but on the sixth day it increases sharply, exceeding normal by 10 times. The inhibitors disappear when the free virus appears; the receptors decrease when the virus accumulates and reaches certain titers. Possibly the variation in the inhibitor level in the bodies of people corresponds to individual variations in their resistance to influenza (Smith, 1951).

The findings of Burnet, Lush and Jackson (1939), who found a virus-inactivating agent in the nasal secretion of certain persons, are apparently associated with tissue reactivity. These persons had a low antibody titer against influenza which did not rise after intranasal immunization with live virus. Their nasal secretion inactivated the influenza virus and certain other viruses. On boiling, this property of the nasal secretions disappeared after 10 minutes, and at a temperature of 36°, in several days. This factor is not lysozyme nor is it an antibody, because there is no specificity of the effect. In contrast to inhibitors this agent inactivates the influenza virus as well as certain other viruses (herpes, louping ill, Rous sarcoma) and is encountered inconstantly in the human nasopharynx.

The significance of local nonspecific immunity to influenza has been pointed out repeatedly by Gamaleya, who presents the observations of Burnet, Lush and Jackson mentioned as a new proof of the justifiability of the principles propounded by him (Gamaleya, 1942).

The phenomenon established in the experiments of Zakstel'skaya (1952) also constitutes one of the local immunity factors. After the intranasal immunization of mice the repeated instillation of the virus after two or three weeks and later is not only unassociated with a multiplication of it but, on the other hand, the virus rapidly dies out. In addition to the role of antibodies (about which more will be said below), this is explained also by the fact that the virus cannot multiply in immune cells not only in vivo but even in the event the lung tissue is washed free of antibodies and is cultivated as an explant. This phenomenon is not observed if the mice have been immunized paranasally: in this case, a multiplication of the virus does occur in the washed-out lung tissue culture.

In connection with the works of Burnet, Lush and Jackson investigations were carried out by Francis and others (1943), who established the fact that the virus-inactivating agent in the nasal mucus may be connected with antibodies. These authors showed that the inactivating factor of the nasal secretion is found in the globulin fraction, is destroyed on heating to 70°; it is not lysozyme and is not present in children under three or four years of age. The virus-inactivating factor accumulates in parallel with the rise in antibody titer in the blood and apparently is related to it. Subcutaneous vaccination against influenza leads to an increase in the inactivating effect of the nasal secretion (Francis, 1942).

This problem has been studied in detail by Solov'yev and Parnes (1944, 1946). It was shown that in influenza patients in the stage of convalescence a simultaneous increase in virus-neutralizing titers occurs in the blood and nasal secretion (Fig. 164). Like antibodies, the nasal secretion factor possesses the property of checking the hemagglutination reaction (Shubladze and Solov'yev, 1943). After immunization of rabbits a parallel increase occurs in the neutralizing activity of the blood and nasal secretion. The virus-neutralizing agent in the nasal secretion is type-specific with respect to the virus with which the rabbits were immunized. It was conclusively shown by these investigations that the neutralization of the virus is conditioned by specific antibodies against the influenza virus. In a number of experiments the authors showed that antibodies are not formed in situ (that is, not in the nasal mucosa) but rather penetrate into it from the blood.

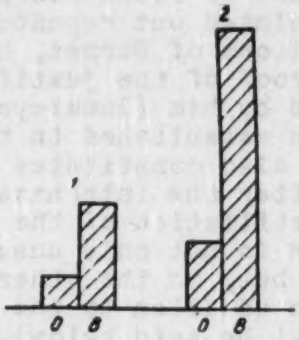

Fig. 164. Neutralizing Titer of Nasal Secretion (1) and Serum (2) of Influenza Patients in the Acute Period (O) and During the Period of Recovery (B) (Solov'yev and Parnes, 1946).

Afterwards, Solov'yev (1950) established the capacity of the lungs for secreting influenzal antibodies when

the lung tissue is cultivated in vitro. The method of the investigation consisted in the repeated immunization of two groups of rats with the PR8 virus, of which one group was injected with the antigen intranasally, and the other, intraperitoneally. After a bleed-out and total irrigation with 300 cubic centimeters of Ringer's solution pieces of lung tissue and splenic tissue were extracted from the animals. The minced tissue was promptly transferred to individual Erlenmeyer flasks containing a nutrient mixture of Tyrode's solution, chicken plasma, chick embryo extract and normal (preliminarily heated) rat serum. The flasks were put into a thermostat at a temperature of 37.5°, and a certain quantity of fluid was extracted from them every day (with the subsequent addition of an equal amount of Tyrode's fluid) for the purpose of determining the specific antibodies. The antibodies were determined by two methods: the hemagglutination-inhibition reaction of chick erythrocytes and the neutralization reaction on mice.

In Fig. 165 the results of these experiments are represented schematically. As seen, after respiratory immunization antibodies were found mostly in the lung tissue and less in the splenic tissue. After pararespiratory immunization the reverse relationships were observed. A decrease in the antibody secretion in the course of time coincided with the process of extinction of the cells in the tissue, which was controlled by histologic investigation.

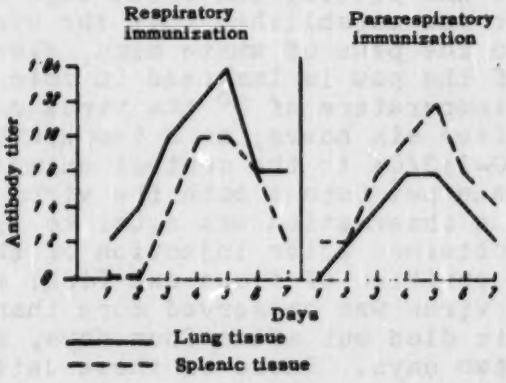

Fig. 165. Formation of Antibodies in the Lungs Depending on the Method of Immunization.

As far as the tissue enzymes are concerned, they do not act on the biologically active influenza virus according to Smorodintsev's and Shishkina's data (1953).

The extinction of the influenza virus and the change in its other biological properties occurred at the same time both in living and dead tissue with inactivated enzymes. Hereby, the temperature coefficient of extinction of the virus (with a temperature increase) ranges within limits of five to eight, that is, it is different from the temperature coefficient of the enzyme reactions, which does not exceed 1.4 to 2.

Therefore, the local immunization factors in influenza are varied, and many of them have not as yet been adequately studied.

55. Mechanism of Destruction of the Influenza Virus in the Body

For the purpose of understanding immunity in influenza the investigations of Smorodintsev, Zil'ber and their colleagues concerning the mechanism of destruction of the influenza virus in the susceptible and resistant organism are important.

Smorodintsev carried out a series of investigations with the aim of studying the mechanisms of destruction of the influenza virus in organisms naturally resistant and immune (Smorodintsev, Shishkina, Zhumatov, 1948-1949). In the first work the authors showed that the influenza virus, when injected subcutaneously into mice and rats and into the brains of mice, dies out in one or two days; this extinction occurs with equal rapidity in vivo and in vitro (killing the animals and putting the focus into a thermostat). In the next work it was established that the virus, after being injected into the paws of white mice, dies out to a different degree if the paw is immersed in cold or hot water. At a bath temperature of 0° the virus remained in a titer of 1:520 after six hours; at a temperature of 45°, in a titer of 1:160-1:320; in the control animals the paws of which had not been put into a bath the virus titer at the same time of the observation was equal to 1:160-1:280. Similar data were obtained after injection of the virus into the abdominal cavities of frogs and fish: at a temperature of 1° the virus was preserved more than five to six days; at 18°, it died out after four days, and at 33-37°, after one to two days. Based on these data, the authors concluded that the cause of the virus destruction in the bodies of resistant animals is the heat effect and, perhaps, oxidative or enzymic processes, which proceed more actively at the body temperature of warm-blooded animals.

Another series of investigations (Smorodintsev, Shishkina, 1940-1947) was devoted to the mechanism of natural and acquired resistance to influenza. These works have

been partially analyzed in the chapter on influenza pathogenesis. The last works of this series showed that in susceptible animals (mice) the virus markedly increases the vascular permeability, and therefore, spreads rapidly throughout the body from the lungs, being destroyed in the internal organs in the next two to four days.

In less resistant animals (rats) the virus does not increase the vascular permeability and, therefore, the bulk of the virus dies out at the site of the injection. The quantity of virus excreted by the kidneys in the urine, in the authors' opinion, is negligible in the total virus balance in the bodies of naturally resistant, immune and susceptible animals.

In recent works, Smorodintsev (Smorodintsev, 1951; Smorodintsev and Shishkina, 1953) returns to this problem and believes that the main cause of death of the influenza virus in the bodies of warm-blooded animals is the direct thermal denaturation of the virus particle under the influence of the normal body temperature of warm-blooded animals.

Zil'ber and others, investigating this matter in two series of published works, came to a different conclusion. In the first series of investigations (Zil'ber, Baydakova, Blank, 1945-1946) the authors studied the influence of the influenza virus on the permeability of the lung tissue and of the kidney. Mice were infected with the influenza virus, and then agglutinating serum was instilled into them intranasally. Normally, the permeability of the lung tissue is low, but it increased markedly in influenza-infected animals, which was measured by the penetration of the agglutinating serum into the blood in considerable quantities and over a longer time than in the controls. In experiments on guinea pigs the subcutaneous injection of the virus increased the permeability of the kidneys to proteins (agglutinating serum was also used as a test), although this effect on guinea pigs was briefer than in mice in the preceding experiments.

In another series of experiments (Zil'ber, Baydakova and Blyumberg, 1946) the authors injected the influenza virus subcutaneously or into the lungs of frogs. After 48-72 hours the virus disappeared from the body; throughout this time it was found in the kidneys. The organs did not possess any viricidal activity. Different results were obtained in experiments on guinea pigs: after the subcutaneous and intranasal injection of the virus small quantities of it were found in the lungs, but it was absent from the kidneys. At this time, no antibodies could have been formed yet, and the organs did not possess viricidal activity. A more detailed study of the fate of the virus in the bodies of guinea pigs showed that it is found in the

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organs in a non-pathogenic form and is excreted in the urine in this form.

It does not seem to us to be very expedient to compare these two viewpoints. Probably both groups of investigators are right. The extinction of the influenza virus in susceptible tissues occurs because of the high temperature, but the excretion of the virus by the kidneys also occurs.

Zakstel'skaya (1953) investigated the urine of influenza patients for its influenza virus content during an epidemic outbreak of 1952. A positive hemagglutination reaction was observed in the urine of two out of 18 adults and in six out of 17 children. In one case, Zakstel'skaya succeeded in isolating the A-prime virus from the urine of children which was similar to strains isolated at this time from the patients' throats. The influenza virus has been isolated from the urine also by Fadeyeva (1953) and Ritova (1953). All these facts are far from being simply chance and constitute evidence of a certain part played by the renal secretory activity in freeing the body of the influenza virus.

56. The Role of the Nervous System in Immunity to Influenza

A study of the role of the nervous system in immunity to influenza has been made, chiefly in recent years.

Zeytlenok and Bychkova (1952, 1953, 1954) attempted to establish the possibilityof conditioned-reflex stimulation of the formation of antibodies to influenza. In experiments on rats the injection of the virus was combined with a mechanical cutaneous conditioned stimulus. The authors succeeded in showing the possibility of producing antibodies under the influence of the conditioned stimulus.

In later experiments they attempted to create, they believe, a cortical dynamic stereotype by means of combining the injection of the virus and pilocarpine. In experiments on rabbits and rats several combinations of the injection of the virus and of pilocarpine were followed by an increase in antibody titer from a single pilocarpine injection alone.

In the experiments of Smirnova, Yakovenko and Rozenberg (1952) the injection of pilocarpine and a moderate vagal stimulation with an induced current during the course of immunization stimulated antibody production against the influenza virus in rabbits; strong stimulation of the vagus nerve depressed their production. Bilateral transection of the cervical sympathetic nerves or stimulation of the cervical sympathetic nerve with an induced current did not affect the antibody formation. The daily injection of

adrenalin affected the animals differently: in some the formation of antibodies was depressed; in others, stimulated. Urethane anaesthesia inhibited antibody formation.

Merkulova (1954) showed that immune animals (rats) not only tolerate toxic doses of the influenza virus well, without dying from the lethal doses but even react differently to them: their conditioned-reflex activity is not much disturbed, whereas in the non-immune animals it suffers severely.

In the experiments of Pille (1954) frequent (up to 60 times) injections of the influenza virus into rats did not lead to any inhibition of antibody production, on the basis of which the author denies Zdrodovskiy's conception of immunological excitation and inhibition. Naturally, these snatchy investigations could not directly explain such a complicated problem as the role of the nervous system in the immunogenesis of influenza. More systematic investigations were made by Kurashvili in Smorodintsev's laboratory.

For the purpose of studying the part of nerve-receptor apparatuses in the immunity to influenza Kurashvili (1953, 1954) in experiments on mice and rats removed part of the femoral nerve on one side, after which he immunized the animals with influenza virus in the denervated extremity. Thereby, he did not find any differences in the antibody titers when the experimental animals were compared with the controls. Novocain block of the sciatic nerve did not affect the production of antibodies either. The same results were obtained through immunization in a denervated skin flap. The author interprets these data as representing the formation of antibodies only when the antigen enters the blood stream.

After the intracerebral injection of the virus the formation of antibodies was much more intense than after intraperitoneal injection of it. A lower degree of resistance of mice to the infection with the virus corresponded to this.

After transection of the spinal cord, the formation of antibodies was depressed; however, at the same time a disorder in heat regulation was observed which led to a reduction in the body temperature of the animals (mice) to 34-36°.

The formation of antibodies in the blood of immunized mice and rats under urethane or veronal anesthesia was decreased compared with that in the blood of control animals (Fig. 166), and the resistance of the body was also lower. Very similar data were obtained from cooling the animals (Fig. 167), as well as through the use of electronarcosis.

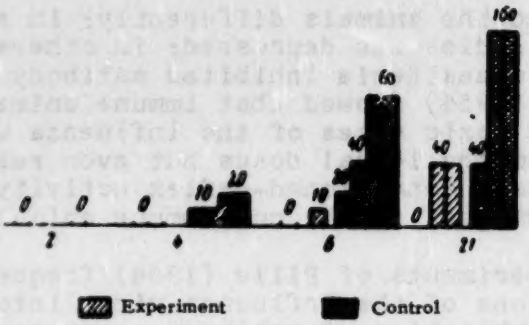

Fig. 166. Dynamics of Production of Virus-Neutralizing Antibodies in Mice Under Veronal Anesthesia.

The figures over the columns represent the serum titer; figures under the columns, the day of the experiment (Kurashvili, 1953).

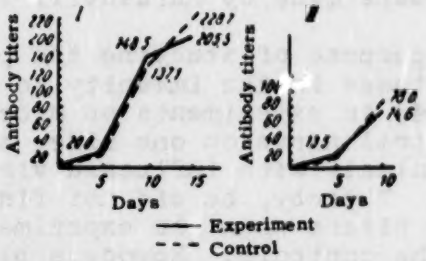

Fig. 167. The Production of Virus-Neutralizing Antibodies in Cooled Animals and Animals Under Anesthesia on the Eighth (I) and 12th (II) Day of the Disease (Kurashvili, 1953).

After immunization of animals under the effect of substances stimulating the nervous system the production of virus-neutralizing antibodies also decreased (Fig. 168). If these substances were injected 2 $^{1}/_{2}$ to 3 months later, when the antibody titer had fallen, the loss of immunity was delayed (Fig. 169).

Therefore, the role of the nervous system as a regulator of the immunological reactions in influenza has been established beyond doubt through these investigations. At the same time, the data presented do not give us the grounds for accepting the theory of reflex production of antibodies in influenza. We can agree with the statements of Ado (1954) that this theory of antibody production represents a mechanical transferrence of the principle of reflexes as the most important form of regulation of the body functions to the immunochemical processes; the conversion of normal into immune globulin requires the direct influence of the antigen on the effector organ (reticuloendothelial system).

---- control; ——— injection of strychnine; —o— injection of morphine; -·-· injection of amphetamine sulfate; ---- injection of caffeine

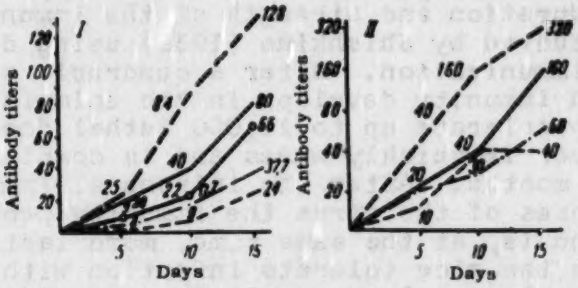

Fig. 168. Production of Virus-Neutralizing Antibodies After Immunization of Mice (I) and Rats (II) With Stimulated Nervous Systems (Kurashvili, 1953).

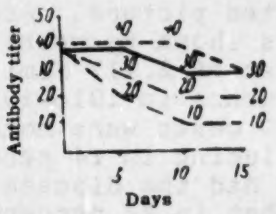

Fig. 169. The Effect of Substances Stimulating the Nervous System on Waning Immunity.

The key is the same as for Fig. 168 (Kurashvili, 1953).

We do not consider it possible to substitute hasty conclusions for the accumulation of experimental naterial, and we believe that in the matter of the role of the nervous system in the production of immunity to influenza we should limit ourselves to the general considerations expressed in the past (Zhdanov, 1952).

57. Certain Characteristics of Immunity in Influenza

A certain idea concerning the duration of immunity is gained from epidemiological observations and experimental data.

In ferrets after intranasal infection a strong immunity to a second influenza virus infection is maintained for three months. After five months part of the animals still maintains a strong immunity; part becomes partly susceptible, carrying reinfection in a mild form (the fever

lasts less than a day). After six to seven months the majority of the animals becomes partly susceptible. After 8 to 12 months, the animals lose their immunity to influenza entirely (Smith, Andrewes and Laidlaw, 1935).

The duration and strength of the immunity in mice have been studied by Shishkina (1939) using different methods of immunization. After a quadruple subcutaneous immunization immunity develops in the animals which permits them to tolerate up to 10,000 lethal doses of the virus; however it quickly wanes and is completely lost after three months. After the intranasal immunization with sublethal doses of the virus the immunity produced is just as strong and is, at the same time, more lasting: after three months the mice tolerate infection with 10 lethal doses of the virus. In general, the immunity of mice to virulent strains of the influenza virus, as in ferrets, does not last particularly long and is measured in months.

Epidemiological observations indicate the greater duration of immunity to influenza in people, although they show quite a variegated picture. According to the data of Vaughn (1922), it was shown by means of a questionnaire given to 10,000 member of 2,117 families that 45 percent of them had had influenza in 1918-1919. During the influenza epidemic of 1920 cases were noted in only 27 percent of the families, including in 14 percent of the families members of which had had the disease in 1918-1919. To this it should be added that in 42 percent of the families there were no cases of influenza in 1918-1919 nor in 1920. In San Quentin Prison in California there were three influenza outbreaks in 1918: in April-May, October and November. Each time, new arrivals or prisoners who had not become sick during the previous outbreaks were affected. The author concludes that immunity in influenza lasts 10-17 months.

These and other data obtained before the discovery of the influenza viruses cannot be considered reliable, because it is not clear whether the new cases were of the same or of different etiology (A or B viruses). According to later data of Sigel and others (1950), second cases of influenza A-prime were observed in school after two years.

In this connection Ritova's observations (1953) on the duration of immunity in children deserve attention. During her observation of 100 children from the time of birth until they were three years of age, she made a detailed study of cases of influenza among them, conducting virological and serological examinations which permitted her to establish the etiology of the cases accurately. In various children repeated cases of influenza-like nature were not uncommonly observed; however, these were acute

catarrhs of the respiratory tract in the majority of cases rather than influenza (Fig. 170) or cases of influenza produced by various pathogens. Cases caused by the same pathogen occurred a second time no earlier than two years after the first (Fig. 171). Subsequently, Ritova (1954) extended her observations to include 558 children, who were under observation for six years. The shortest interval after which a case of A-prime influenza was repeated was two years and 15 days; the majority of the children became sick a second time after three years. Correspondingly, among 220 children who had had influenza B, the shortest time interval before a second case of it occurred was two years and nine months; in the others the interval was longer. Apparently, in the majority of persons immunity to influenza A and A-prime lasts about two or three years; to influenza B, somewhat longer (three to five years).

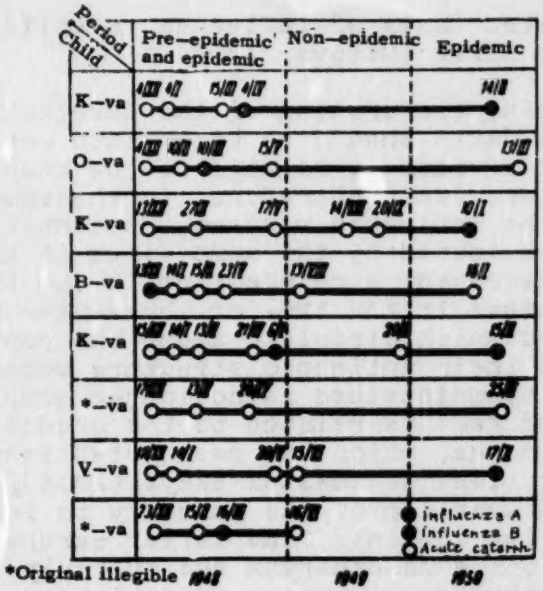

Fig. 170. Incidence of Recurrent "Influenza" in Children According to Clinical Symptoms (Ritova, 1953).

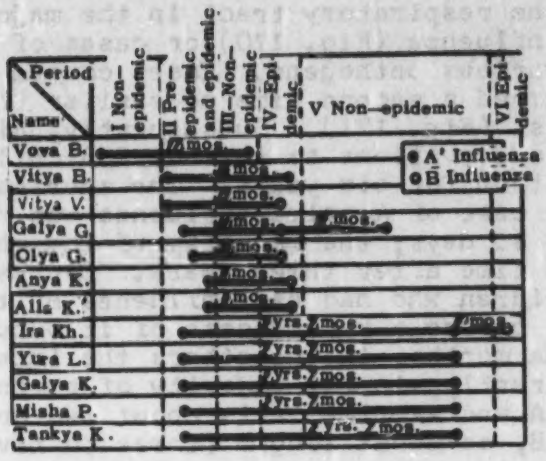

Fig. 171. Repeated Cases of Influenza in Children Observed Since Birth (Ritova, 1953).

In analyzing the problem of the duration of immunity in influenza two facts should be taken into consideration.

The first of them is related to the change in the antigenic structure (and, therefore, in the immunological properties) of the influenza viruses. Essentially, each new repeated case caused by the same virus (A and A-prime or B) does not represent a reinfection in the full sense of the word, because in the two- or three-year period the influenza viruses which circulate among the population manage to change their antigenic structure somewhat. Therefore, the immunity maintained is no longer complete.

The second fact is related to the gradual loss of immunity to influenza, which has been established by the special investigations of Zakstel'skaya (1954), who studied the strength and duration of the immunity to influenza produced by vaccine strains. The latter survive after introduction into the nasopharynx and multiply in the tissues of the nasopharyngeal mucosa producing an immunological reaction of the body. A second nasopharyngeal instillation of the same virus strain into volunteers after two or three weeks does not lead to the survival and multiplication of the virus; the latter dies out and is no longer found in the next few hours. The immunity lasts in this state for eight to ten months in the majority of the persons inoculated (with variations within broad limits), after which it is gradually lost, and the instillation of the virus again is associated with its multiplication in the nasopharynx.

Therefore, it should be considered that natural immunity in influenza is also lost gradually after having the disease. Immediately after the disease the strongest immunity is created, which excludes the possibility of virus multiplication in the body. This degree of strength of the immunity is apparently brief and is maintained for no more than a year. After the loss of the immunity to the infection, however, the immunity to the disease remains, wherein multiplication of the virus occurs in the body in the form of an asymptomatic infection without any clinical signs of the disease. The existence of the asymptomatic infection in influenza has been proved at the present time, about which more will be said in the chapter on influenza epidemiology. This state apparently lasts two or three years in influenza A and A-prime and three to five years in influenza B, after which a complete loss of immunity to influenza occurs, and the organism reacts with disease in response to infection.

The nature of the immunity in influenza depends on the routes of administration of the virus into the body. In the old investigations (see, for example, Andrewes and Smith, 1937), it was noted that immunization through the respiratory tract creates a better immunity to influenza than subcutaneous or intraperitoneal immunization. Shishkina's data (1940) have been presented above; she graphically illustrated this feature of the production of immunity to influenza. This author compared the efficacy of various methods of immunization of mice against influenza: intranasal, inhalation (with sublethal doses), intraperitoneal and subcutaneous. In the last two methods the mice were immunized with doses which considerably exceeded the lethal doses after the virus was introduced into the respiratory tract.

These experiments clearly show that the introduction of the virus into the respiratory tract produced a much stronger immunity than immunization by pararespiratory methods. However, at that time there were no avirulent strains of influenza virus in the laboratories and, therefore, immunization through the respiratory tract even with sublethal doses was always followed by death of a considerable number of the animals.

Similar data were obtained later, when it became possible to work with strains avirulent for mice (Bruyn and others, 1949; Bowers and others, 1952). This problem was studied in particular detail in the USSR.

Karakuyumchan (1950) immunized mice with killed and live virus by various methods and studied the concentration of antihemagglutinins and virus-neutralizing antibodies in the blood. The interrelationship of them depended on the

method of immunization as well as on whether live or formalinized virus had been used for the immunization (Table 75).

Table 75

Antibody Titers Depending on the Method of Immunization

Method of immunization	Virus	Antihemagglutinin titer	Titer of neutralizing antibodies	Index of resistance
Intranasal	Live	40	24 000	22 200
	Formalinized	20	20	
Subcutaneous	Live	128	18 600	691
	Formalinized	240	3 160	
Intraperitoneal	Live	480	54 000	4 990
	Formalinized	560	22 500	

In experiments with the repeated injection of sublethal doses of the virus Karakuyumchan (1950) observed the death rate of the animals immunized with the live and killed virus. Thus, mice immunized with the live virus intranasally died in 27 percent of the cases; subcutaneously, in 38.3 percent of the cases; intraperitoneally, in 30 percent; the mortality rate after immunization with the killed virus was expressed, respectively, as 200, 100 and 40 percent.

In the works of Kravchenko and Sekretta (1950) it was established that after the injection of mice with a non-lethal dose of the influenza virus it multiplies in their lungs but does not show any clinical manifestations of the disease, and the animals do not form any immunity to subsequent infection with the influenza virus. A very weak immunity is created to subsequent infection with the influenza virus in mice following a double intranasal instillation of 1/100 MLD of the influenza virus. A strong immunity to the influenza virus may be obtained in mice after they are inoculated with 1/100 and 1/10 MLD of the influenza virus at an interval of 7 to 15 days; this effect is also obtained if 1/10 is first given, and then 1/100 MLD.

It has been established that in mice immunized with the live influenza virus, the antibody titer in the blood is not an index of immunity.

According to Sekretta's data (1950), the rabbits and mice react differently to different routes of administration of the influenza virus: the sera of rabbits immunized subcutaneously, intracerebrally and intranasally possess the same virus-neutralizing power, whereas in mice the sera

after intranasal immunization are more active than after subcutaneous and intracerebral immunization.

The production of immunity by the intranasal method of immunization has its own characteristic features and occurs in two phases. The first phase of immunity, which occurs immediately after the injection of the vaccine, is due to a blockage of the susceptible cells by the influenzal antigen and does not depend on the antibodies. Factors of humoral and tissue immunity (Sokolov and Kulikova, 1950) underlie the second phase of resistance, which occurs after 7-10 days.

In Table 76, data are presented from the work of Sokolov, Shimshelevich and Kulikova (1951) wherein intranasal and subcutaneous immunization was conducted with the same doses of concentrated formalinized virus.

Table 76

The Efficacy of Intranasal and Subcutaneous Immunization of Mice With Formalinized Virus

Infecting Dose LD_{50}	Method of immunization	Quantity of Vaccine Inoculated in cubic centimeters		
		0.05	0.005	0.0005
1	Intranasal	0/20	0/20	4/20
	Subcutaneous	0/20	0/20	8/20
10	Intranasal	0/20	10/20	9/20
	Subcutaneous	2/20	12/20	16/20
100	Intranasal	11/20	18/20	20/20
	Subcutaneous	10/20	18/20	20/20

Key: Numerator -- the number of mice which died;
denominator -- the number of infected mice.

In the experiments of Sokolov (1954) the intranasal immunization of mice with live virus in a dose of 0.1 LD_{50} produced immunity to 1000 or more LD_{50} of the virus; immunization in a dose of 0.01 LD_{50}, to 100-1000 LD_{50} of the virus; at the same time, the concentrated and formalin-killed virus in a dose of 0.05 cc produced an immunity to less than 100 LD_{50}, while in subcutaneous immunization one cubic centimeter of the concentrated killed virus had to be injected to obtain an immunity to 100 LD_{50}.

Special investigations on the significance of the site of inoculation of the influenza virus in the creation of immunity were made by Zakstel'skaya (1953). The author

immunized mice subcutaneously and intranasally with a non-pathogenic strain of the A-prime virus (Klim), and after three weeks inoculated this virus into immunized mice intranasally. Two groups of mice were immunized with an influenza A-prime virus strain (Klim) non-pathogenic for them; one group subcutaneously twice with 0.2 cubic centimeter of allantoic fluid and the other group, twice with 0.00005 cubic centimeter, calculating for undiluted allantoic fluid. After three weeks, both groups of mice were infected intranasally by the same virus strain in different doses. The presence or absence of virus multiplication in the lungs of immune mice measured quantitatively and dynamically served as the index of immunity. The results of one of the experiments are presented in Fig. 172.

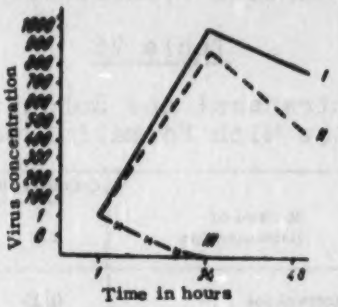

Fig. 172. Influenza Virus Multiplication in Lungs of Mice Immunized Twice by Different Methods.

I -- control; II -- subcutaneous immunization; III -- intranasal immunization; the concentration of virus an hour after the infection is taken as one unit (Zakstel'skaya, 1953).

On the basis of repeated investigations it was established that after a single subcutaneous immunization a very weak immunity was created; the subsequent intranasal infection with 500-1000 LD_{50} of the virus was followed by just as active a multiplication as in the control. The double subcutaneous injection of the virus increased the immunity, but in this case also its multiplication occurred after infection with 500-1000 LD_{50} of the virus, although the duration of time during which the virus was isolated and the height of its titer curve were less than in the control.

Completely different results were obtained after the intranasal immunization. Even a single immunization created a good immunity; infection with 500-1000 LD_{50} of the virus was not associated with virus multiplication in the lungs. After a double immunization the subsequent infection with even 1000 LD_{50} of the virus was not accompanied by multi-

plication of it in the lungs.

Therefore, the intranasal immunization conferred a strong immunity against virus multiplication in the lungs, although the immunizing dose was 4000 times less than after subcutaneous immunization, which produced a negligible immunity to virus multiplication in the lungs. For the purpose of studying the causes of these differences investigations were made of the concentration of antibodies in the blood and tracheobronchial secretion, as well as of the tissue immunity. Thereby, it was established that the concentration of antihemagglutinins and of virus-neutralizing antibodies in the blood was approximately the same in the mice of both groups. At the same time, the neutralizing power of the tracheobronchial secretion in the intranasally immunized mice was 100-500 times greater than in the subcutaneously immunized animals. The multiplication of the virus in eluted lung tissue was studied as a test for the evaluation of the tissue immunity. In tissue cultures of the lungs of control mice the virus survived for 72-96 hours and multiplied; in tissue cultures of mice immunized subcutaneously the virus also survived for 72 hours and multiplied to some extent; in tissue cultures of mice immunized intranasally the virus did not multiply and died out in the next few hours (only in one of the four experiments was it preserved for a day).

Similar observations on vaccinated volunteers showed very similar results. The subcutaneous immunization did not prevent the virus multiplication in the nasopharynx when it was inoculated after two or three weeks, whereas intranasal immunization eliminated the possibility of virus multiplication when it was introduced a second time into the nasopharynx. These data will be discussed in greater detail below.

These experiments showed that the strongest immunity to influenza is created from the injection of the virus by the route which is natural for this infection, that is, intranasally. In this case an immunity to infection develops which is composed of humoral immunity (blood antibodies), tissue immunity (resistance of lung tissue to virus multiplication), barrier immunity (concentration of antibodies in the nasal secretion) and, apparently, of a number of other immunity factors. After subcutaneous injection the virus figures chiefly as an antigen rather than an infective agent; therefore, the immunity to the antigen elaborated is chiefly humoral (antibodies in the blood). From these experiments it is seen that the humoral immunity is not the decisive factor in the resistance of the body to influenzal infection. Finally, these experiments indicate, first, the regulatory role of the central nervous system in

the production of immunity (otherwise it would be impossible to understand the redistribution of antibodies between blood and nasal secretion which depends on the route of administration of the virus), and, secondly, the natural-historically constituted types of reaction of the body which depend on the nature of the interrelationships between the virus and the macroorganism (in the given case, the pneumotropism of the virus which determines the best form of immunity as intrapulmonary inoculation of the virus rather than a different route).

During the course of study of the immunity in influenza certain additional factors were clarified which have an influence on the production of immunity, namely, the effect of nonspecific stimuli.

According to Friedewald's (1944) data, the addition of killed bacteria (Mycobacterium tuberculosis, M. butyricum) emulsified in paraffin oil or a mixture of beeswax, paraffin oil and lanolin oxycholesterols to a virus suspension markedly increases the immunogenic properties of the virus. Thus, in experiments with the PR8 virus mice immunized once subcutaneously were resistant to 100 lethal doses after a week; if, however, the virus was injected with the adjuvant mentioned the mice became immune to 1,000,000 lethal doses. When rabbits were immunized with the virus plus the adjuvant the antibody titer was 10 times as great as after immunization with the virus alone.

Similar data were obtained by Chalkina (1949) and then by Orlova (1950, 1953), who showed the possibility of increasing the immunogenic properties of formalinized virus by precipitating it with calcium phosphate (the author for some reason calls the adsorbent "a stimulator-precipitant").

By comparing the effect of biological (cheese bacillus) and chemical (calcium phosphate) stimulators in experiments on mice, Orlova (1950) showed that the former increase the immunogenic properties of the influenza virus by 4 1/2 to 5 times; the latter, by 10-15 times.

Belkina (1954) studied the effect of nonspecific stimuli on the immunizing efficacy of live influenza vaccine (used intranasally) in experiments on mice. The simultaneous subcutaneous injection of the NIISI [Scientific Research Institute of Vaccines and Sera] polyvaccine or the injection of it after 48 hours weakened the formation of immunity to influenza; if this polyvaccine was injected a week after the influenza vaccine the immunizing effect of the latter was increased, particularly if the polyvaccine was diluted in physiological solution and instilled intranasally. Similar results have been obtained in experiments with the intranasal instillation of bile. A preparation of aloe, on the other hand, stimulated the

immunity to influenza when it was given seven days before the vaccination.

An unusual characteristic of immunity development in influenza was described by Fazekas de St. Groth and others (1950-1951). The intranasal instillation of the B virus simultaneous with intraperitoneal immunization of mice with the A virus markedly increases (by 100 times) the immunity to the A virus. This phenomenon is also observed where the A virus is introduced intranasally simultaneously with intraperitoneal immunization with the B virus. This phenomenon was called "pathotopic potentiation" by the author, because the immunity is increased by the heterologous virus when inoculated into the respiratory tract. On subsequent investigation it was shown that after the injection of the heterologous virus the antibody level to the virus being tested increases markedly in the respiratory tract, remaining unchanged in the blood. Subsequently, it was shown that certain substances act similarly when they are introduced into the respiratory tract: vaccinia virus, metaperiodate, tannic acid, zinc sulfate. The inoculation of these substances by themselves does not increase the immunity to influenza.

It must be supposed that in this case the injection of the influenza virus into the abdominal cavity and the simultaneous stimulation of the respiratory tract stimulate the natural influenzal infection (virus infection in the respiratory tract) to a certain degree. It seems to us that this phenomenon constitutes evidence of the role of the nervous system in the production of immunity to influenza, and we are inclined to consider it in this sense, as was stated in the generalization of Zakstel'skaya's material on the characteristics of immunity after the virus is introduced into the repiratory passages.

As a conclusion to this section it is fitting to present the data of Zakstel'skaya (1953) on the change in the various immunity indices after immunization of animals. A group of mice was vaccinated once with formalinized A virus (Shklyaver), after which various immunity indices were tested for 100 days. As seen from Fig. 173, a parallelism is observed, in general, in the change in the intensity of various immunity indices to influenza. Ten days after the immunization in mice all the immunity indices studied -- the concentration of antibodies in the blood, the resistance of the tissues to the toxic effect of the virus, the resistance of animals to infection and intoxication -- are high. They remain in this condition for less than two months, after which an almost simultaneous gradual decrease occurs in them. After 100 days all the indices drop markedly and approach the initial level prior to

immunization. These data provide a basis for using various immunological indices for evaluating the strength of immunity to influenza.

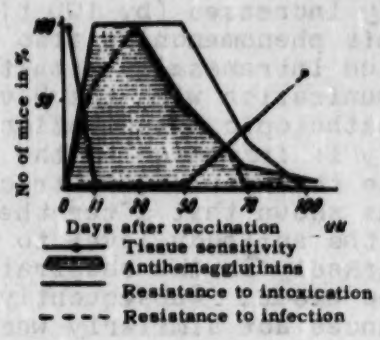

Fig. 173. Change in the Various Indices of Influenza Immunity in Mice in the Course of 100 Days After a Single Vaccination (Zakstel'skaya, 1953).

58. Experimental Basis for Active and Passive Immunization Against Influenza

The study of the immunity in influenza was the basis for the development of research on active and passive immunization against this infection.

The investigations on active immunization against influenza, directed toward a search for effective vaccines, developed in two main directions: the obtaining of killed vaccines for subcutaneous immunization and the obtaining of live attenuated vaccines for immunization by the respiratory tract. The former trend is to date being developed chiefly abroad; the latter, in the USSR.

After it was shown that the subcutaneous injection of live virus into mice does not produce the disease in them but is associated with the development of immunity to intranasal infection (Andrewes and others, 1934) numerous attempts were made to produce immunity in mice by means of vaccination of them with formalinized virus from mouse lungs.

The first attempts to produce an artificial immunity to influenza in mice by means of immunization with formalin-treated lung filtrates of mice infected with influenza gave negative results (Smith, Andrewes and Stuart-Harris, 1937; Stuart-Harris, 1938-1939; Taylor and Dreguss, 1940). How-

ever, after unfiltered material began to be used (because it was made clear that considerable virus losses occur) more satisfactory results were obtained.

Smith and others (1935) produced an immunity to intranasal infection in mice and ferrets by means of subcutaneous immunization of the animals not only with live but also with formalin-killed virus. In the blood of the vaccinated animals virus-neutralizing antibodies appeared, and a partial resistance to infection was produced. Fairbrother and Hoyle (1937) obtained a similar result with virus killed by heating at 57° for 30 minutes. In the experiments of Andrewes and Smith (1937) virus which had been purified by means of washing in colloidal sacs and rendered innocuous with formalin produced an immunity in mice to intranasal infection with live virus after subcutaneous injection; however, the purified virus was less stable than the virus in the mouse-lung suspension.

In Shishkina's experiments (1938) it was established that it is possible to create quite a high degree of resistance of the animals to influenzal infection by means of the repeated injection of adequate doses of the formalinized virus.

In contrast to the live virus the formalinized virus did not cause much mortality in mice. Similar data were obtained by Chalkina (1940) in ferrets.

Orlova (1943) in experiments on animals showed the possibility of producing an immunity after vaccination with a formalinized influenza virus in a mixture with typhoid-paratyphoid vaccine, tetanus toxoid or typhus vaccine. The immunity was of the same strength as after immunization with each component separately.

Development of the methods of cultivating influenza viruses in chick embryos made it possible to replace mouse lungs by allantoic fluid as the source of the influenza virus. Immunization with allantoic formalinized cultures of the virus proved to be just as effective as immunization with a lung suspension of mice infected with influenza virus (Francis and others, 1943). The purification and concentration of the virus was an easier task with this method. Purification was accomplished by means of centrifugation (Stanley, 1945) and elution on Elford filters (Chalkina, 1948). Chalkina showed that with various methods of purifying the influenza virus (supercentrifugation, elution on Elford filters) its immunogenic properties are maintained to an extent proportional to the quantity of active virus. The gentlest method of inactivating the virus is the addition of small quantities of formalin.

All these methods of purification and concentration of the influenza viruses were soon replaced by erythrocyte

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adsorption, however. In Sokolov's experiments (1949) a polyvalent vaccine prepared by this method from A and B viruses showed definite effectiveness for mice, protecting them from 1000 LD_{50} of the A virus and from 100 LD_{50} of the B virus (Table 77).

Table 77

Immunogenic Properties of Polyvalent Vaccine in Mouse Experiments

Virus titer with which mice were infected	Group of mice	Survival rate of mice after infection with the virus in a dilution of									ID_{50}	Antibody titer
		10	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}		
A (PR8)	Immunized	.	.	.	10/10	0/10	0/10	0/10	.	.	$10^{-3.8}$	1:160
	Control	.	.	.	.	.	10/10	8/10	5/10	0/10	10^{-7}	
B (Lee)	Immunized	6/10	0/10	0/10	.	.	.	.	.	.	10	1:40
	Control	.	.	10/10	5/10	0/10	.	.	.	.	10^{-3}	

Key: The numerator represents the number of mice which died; the denominator, the number of infected mice; . not infected.

The minimum immunizing dose was equal to 10^{-4} for the A virus and 10^{-2} for the B virus when the mice were infected with 10 LD_{50} of the corresponding viruses.

There is no need to list the numerous investigations on animals wherein a basis was given to the use of killed vaccines for subcutaneous immunization against influenza. It is important to note that the idea itself of subcutaneous vaccination against influenza could not be extensively realized in practice, because, first of all, subcutaneous immunization with the killed virus does not create such a strong immunity as immunization with the live virus through the respiratory tract; secondly, the absolutely essential increase in the virus concentration in the vaccine to achieve a strong immunity involves an increased reactivity and an increase in the cost of the vaccine; thirdly, subcutaneous immunization of millions of people against such

an infection as influenza is difficult in practice. By virtue of these facts investigations on influenza immunization through the respiratory tract with live attenuated viruses have obtained considerable development in the USSR.

The possibility of intranasal immunization against influenza with live attenuated virus was given a basis in experiments on white mice, white rats and ferrets in 1937-1938 by Smorodintsev, Shishkina and Chalkina (Shishkina, 1938; Chalkina, 1940; Smorodintsev and Chalkina, 1953). In Shishkina's experiments the virus was injected in a mixture with immune serum, or the injection of the latter preceded the immunization of the animals with the virus. Under these conditions, the mice were resistant to subsequent infection with a large number (up to 20,000 lethal doses of the virus).

Almost simultaneously Burnet's investigations were published (1937); he established the fact that allantoic cultures of the influenza virus, while not very pathogenic for mice and ferrets, produce an immunity after intranasal inoculation to large doses of the virulent strains of the virus. Thus, for example, the single inoculation of an allantoic culture diluted 1:10 protected mice after 7 to 20 days against 100 LD_{50} of the virulent strain. Similar data have been obtained by Andrewes and Smith (1939).

Afterwards, Sokolov (1949, 1954) showed that a definite immunological effect can be obtained also through the intranasal administration of concentrated formalin-killed virus. His data on the immunogenicity of the formol vaccine have been presented above.

In the intranasal administration of this killed vaccine the immunizing dose, which protected 50 percent of the mice against 100 lethal doses of the virus, was equal to 0.05 cubic centimeter, that is, it was 20 times less than after paranasal immunization.

In Fig. 174 data are presented graphically concerning the dynamics of development of immunity. The first phase, which lasted two or three days, is associated with interference; afterwards, immunity develops which is associated with antibodies and other specific factors.

It is natural, however, that these investigations were not developed much, because the immunity produced by the intranasal inoculation of killed virus cannot be compared with that produced by the live virus.

The subsequent development of research on active immunization against influenza with live attenuated viruses, particularly after observations were developed on the epidemiological efficacy of vaccines, were directed at overcoming the difficulties associated with obtaining avirulent and highly immunogenic strains. The theoretical basis of

the methods of obtaining live influenzal vaccines and the difficulties which were encountered thereby have been analyzed in the chapters on variability, pathogenesis and in this chapter; therefore, there is no need to return to them, particularly since this problem will be discussed in the chapter on prophylaxis.

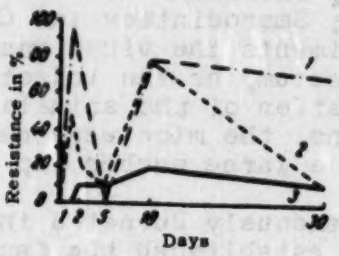

Fig. 174. Dynamics of Development of Immunity in Mice Inoculated Intranasally With Formol Vaccine.

Key: 1 -- resistance to 10 LD_{50}; 2 -- resistance to 100 LD_{50}; 3 -- antibody concentration (Sokolov, 1954).

Investigations on passive immunization against influenza have been comparatively few. In the USSR this problem has been worked on by Smorodintsev and his co-workers (Smorodintsev, Shishkina, 1938; Smorodintsev, Gulamov and Chalkina, 1940). In the first of the works mentioned it was shown that the inoculation of specific influenza antiserum into the respiratory tract of mice exerted a distinct therapeutic effect when it was used early. The effect of the serum was much less pronounced if it was injected intraperitoneally or subcutaneously. The prophylactic effect of the serum was even more pronounced, and it gave an indubitable effect even when it was administered 10 days before infection with the influenza virus. It was shown through special investigations that antibodies introduced into the respiratory passages remain there for a long time, and the prophylactic effect of the serum is associated with this. Similar data have been obtained abroad in experiments on ferrets by Laidlaw and co-authors (1935), Zellat and Henle (1941), and in experiments on mice by Vieuchange (1940) and Henle and co-authors (1941).

The further development of the research on passive immunization against influenza was associated with the use of serum therapy and serum prophylaxis of influenza in people.

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SIXTH CHAPTER

CLINICAL ASPECTS

59. Introduction. Brief Historical Information

In presenting the clinical aspects of influenza (of virus nature) we should like in passing to touch on the acute (colds) catarrhs of the respiratory tract, comparing one disease with the other. This is necessary for better understanding of the characteristic features of the clinical course of influenza, since there are many similarities between it and acute catarrhs, and these diseases are quite difficult to differentiate from each other.

The difference between these two diseases was pointed out for the first time in Russian medicine by the St. Petersburg Professor, Ekk. In his lectures, which were published in 1852, he draws a parallel between influenza and epidemic catarrh.

N. Filatov adhered to the same opinion. In "Lectures on the Acute Infectious Diseases in Children" (1885) he writes: "apparently, authors do not recognize any differences between influenza and simple bronchitis, except for the epidemic nature of the former, but this view is decidedly untrue, and I shall attempt to show that the difference between these two diseases is very essential and is expressed not only in the etiology but also in the clinical course". Filatov gave a detailed description of the clinical picture of influenza and of acute catarrhs.

In the clinical lectures which were published in 1903, Filatov distinguishes between the two different forms of influenza even more definitely: one of them -- childhood influenza -- is accompanied by catarrhal signs of the upper respiratory tract with which the disease begins. The coryza and dry cough observed here frequently precede the occurrence of fever. The other form is influenza with general signs. The cough and coryza occur later and are not most prominent.

We find a full support of Filatov's viewpoint on the nature of these diseases and the terminology which he used

in the work of Kuznetsov and German (1890) as well as in Hecker (1923). The latter's indication that the diagnosis of influenza "is not always easy and requires exact clinical observation on the part of the physician," because otherwise the most varied diseases may be classified under the rubric "influenza" is interesting.

In Hecker we first encounter the term "seasonal catarrh," which in the 1930's was used and popularized by Smorodintsev (1937, 1938, 1939, 1948, 1949) and Rossiyskiy (1937, 1939, 1945, 1947, 1948) after mention of this term by Bashenin in his work in 1926. The term "seasonal catarrhs" was afterwards discarded by Soviet physicians.

Of the number of works in which a description of the clinical picture of influenza and of acute catarrhs is presented the works of the English investigators Stuart-Harris co-authors (1938) deserve attention; they used virological methods of investigation for making the diagnosis. They were the first in the literature to present a differential diagnostic table of symptoms of influenza and acute catarrhs of the respiratory tract ("febrile catarrhs" according to their terminology) (Table 78).

Table 78

Differential Diagnosis of Influenza and Febrile Catarrhs

Feature	Influenza	Febrile catarrhs
onset	acute	gradual
symptoms	chiefly general	mostly local (in the respiratory organs)
cough	dry, short	in theform of paroxysms, moist
voice	husky	hoarse
fever	frequently biphasic	rarely biphasic
pharynx	posterior pharyngitis	tonsillitis and pharyngitis
complications	bronchiolitis and pneumonia	bronchitis and bronchopneumonia
epidemic	brief with sharp peak (rapid rise)	increasing slowly and drawn out
clinical picture	uniform but of different degrees of severity	various, frequently with diseases of the tonsils.
blood	not typical	not typical
virus	isolated from the throat	does not participate

Although the data of Table 78 have many defects, for esample, indication of the atypicality of the blood picture does not correspond to the facts, nevertheless they have served as an impetus for making more thorough investigations in this direction.

Simultaneously with the observations of the English authors a thorough study was made of the clinical aspects of influenza and acute catarrhs of the respiratory tract by virologists (Smorodintsev and others, 1936-1938) and clinicians (Tushinskiy, 1936-1939; Korovin, 1937, and others) in Leningrad. They made comprehensive observations on experimental influenza and acute catarrhs, studying at the same time the virological, bacteriological, clinical and hematological data in both types of diseases. In connection with the organization of the department of viruses of the VIEM [All-Union Institute of Experimental Medicine] in Moscow and with the opening of an influenza department in the internal medical clinic of the VIEM in 1938 joint work was accomplished by clinicians (Epshteyn and coworkers) and virologists (Smorodintsev, Solov'yev, Zhdanov and coworkers). The clinical observations were continued in Leningrad (Tushinskiy and others), and after the organization of Institute of Infectious Diseases of the Academy of Medical Sciences USSR in 1949, in Kiev as well (Zyukov, Khomenko, Morozkin and others). In 1949, the study of the clinical aspects of influenza in Leningrad (Tushinskiy, Kofman, Davidenkov, Undrits) was increased. It was carried out conjointly with the department of viruses of the Institute of Experimental Medicine (Smorodintsev and others) and the Institute imeni Pasteur (Romanenko and others).

In sum, the clinical aspects of influenza have been studied in greater detail at the present time owing to the use of virological and clinical-laboratory methods of investigation.

60. Clinical Characterization of Diseases Erroneously Taken for Influenza

The discovery of the specific pathogen of influenza made it possible to distinguish true influenza from a mass of other diseases clinically similar to it, and thereby to make the clinical characterization of it more precise.

The everyday study of the clinical picture of diseases in persons sent to the hospital with the diagnosis of influenza over the course of a number of years has shown that a considerable percentage of these diseases etiologically has nothing in common with influenza, and that the diagnosis of them was made incorrectly. Of 600 patients whom we observed from January through June 1942 in the Hos-

pital of Infectious Diseases of the Second Moscow Medical Institute (during the period of evacuation into Omsk) influenza was diagnosed correctly in 321 (53.5 percent) and incorrectly in 279 (46.5 percent). As an illustration we are presenting Table 79).

Table 79

Different Diseases Erroneously Considered Influenza

Diagnosis	No of patients	Percent
Pneumonias (different), pulmonary abscess, pleurisy	134	52,0
Pulmonary emphysema, chronic bronchitis	11	
Pulmonary tuberculosis (16) and tuberculosis of the lymph nodes (1)	17	6,1
Various sore throats (10), antral sinusitis (2)	12	4,3
Typhus (23), typhoid fever (11), Paratyphoid B (5)	39	17,5
Measles (3), spinal meningitis (1), mumps (1)	5	
Erysipelas (3), brucellosis (2)	5	
Malaria	30	10,8
Dysentery (13), enterocolitis, gastroenterocolitis (5)	18	6,5
Paranephritis (1), thrombophlebitis (1), abscess of the foot (2), mastoiditis (1)	5	2,8
Rheumatic fever (1), urticaria (1), inoculation reaction (1)	3	
Total	279	100,0

From Table 79 is seen that the greatest number of errors (52 percent) occurred in the case of various diseases of the respiratory organs. Cases of tuberculosis comprised 6.1 percent of the errors; acute infectious diseases (typhus and typhoid, brucellosis, measles, erysipelas, and others) -- 17.5 percent; malaria -- 10.8 percent; acute diseases of the gastrointestinal tract and dysentery -- 6.5 percent. Errors were also noted in diagnosing rheumatic fever and surgical diseases.

The errors in diagnosing influenza were committed not only in Omsk where the observations were made but also in other large centers in the Soviet Union as well as abroad. This can be illustrated by Table 80.

Table 80

Virus Diseases Erroneously Considered Influenza

Diagnosis	No of patients	Percent
Lung diseases, diseases of the bronchi and pleura (42), tuberculosis (13)	55	20,0
ginusitis (40), chronic rhinitis (3)	43	15,0
Sore throat (chronic tonsillitis)	91	32,0
Otitis	2	1,0
Malaria	63	23,0
Surgical diseases	6	4,0
Rheumatic fever, arthralgia	4	
Dysentery	6	5,0
Measles, scarlet fever, typhoid fever, erysipelas	7	
Fever of undetermined origin	2	
Total . . .	279	100,0

In Table 80 data are presented obtained in Moscow concerning the errors made in the diagnosis of influenza during the period from 1938 to 1941. Among 952 patients sent into the hospital of the VIEM with a diagnosis of influenza an erroneous diagnosis was made in 279, which comprised almost 30 percent of the errors. These errors were not much different from those mentioned in Table 79. The difference was only quantitative but not qualitative. In Table 80 chiefly respiratory diseases are involved. Here acute infections are also noted, aside from brucellosis, which is rare in Moscow usually. Malarie in Moscow was observed in a higher percentage of cases than in Omsk. Dysentery, rheumatic fever and various surgical diseases were encountered which were erroneously considered to be influenza.

Diagnostic errors with respect to influenza were noted by various authors before us both in the Soviet Union and abroad, which can be ascertained by examining Table 81.

Sometimes catarrhs of the respiratory tract are taken for influenza, as well as catarrhs which have been produced by chemical or mechanical agents -- dust, gases and others.

In persons suffering from chronic diseases of respiratory organs, throat, nasal sinuses (rhinitis, nasal polyps or deviation of the nasal septum, sinusitis, tonsillitis and others), certain symptoms (coryza, pain on swallowing, cough) accompanying these diseases are usually taken for "the main" symptoms of influenza. Sometimes, dysentery or acute gastrointestinal diseases are considered to be influenza, because the physicians associate them with the gastrointestinal form of influenza about which they have heard while they were still students. Most often, it occurs in the presence of a febrile reaction in persons suffering from chronic but not from acute catarrhal disease of the respiratory tract. This is how the diagnosis of influenza is made, sometimes with an interpretation of it as a gastrointestinal or toxic form, etc. Less frequently, the diagnosis of influenza is made erroneously in cases of different noninfectious and infectious diseases in the absence of catarrhal signs and in the presence of a toxic state.

Table 81

Influenza and Other Diseases With the Same Diagnosis

Author and year	No of patients admitted to the hospital	Diagnosis		Percentage of erroneous diagnoses
		Influenza	Other diseases	
Shteynshneyder, Ratner and Yakobson, 1934	55	28	27	49
Barkhash and co-authors, 1934	100	63	37	37
Lapp and Wicke, 1935	344	269	75	22
Korovin, 1937	96	58	38	40

Dormidontov (1945, 1946) describes influenza-like conditions of allergic origin in infants, for example, after vaccination, parenteral and enteral administration of protein, in tuberculosis or in the process of absorption of pneumonic foci, of pleural purulent exudates and others. Since we do not have any personal experience in this matter we are withholding any statements on these observations, particularly since not all the cases presented by the author are convincing enough so that we can agree with him.

Davydovskiy (1938, 1939) also points out that in tuberculosis or during the absorption of tissue decomposi-

tion products a reaction may be observed like that which occurs in influenza, which is confirmed by Dormidontov's observations.

These, in general, are the various diseases which figure in the diagnosis of influenza.

Under certain conditions errors of the reverse type are observed. Patients with influenza are sent into the infectious disease department with various diagnoses, depending on the infection that the physician has most often encountered during one period or another.

Among them the following diagnoses are involved: febrile state, pneumonia, malaria, sore throat, gastroenteritis and others. Sometimes the physicians, while they have not as yet diagnosed the disease, term it influenza in a purely formal manner in order to make some specific diagnosis afterwards.

In our material we have obtained approximately the same picture, reflecting the specific importance of diseases of the respiratory tract, particularly of the upper respiratory tract, and of various infectious diseases (dysentery, etc.) in diagnosing influenza incorrectly; here, the seasonal local factors have some influence also (period of increase of pneumonias, malaria, intestinal infections, etc.). For example, of 212 patients sent to us in January-February 1941 the diagnosis of influenza was not confirmed in 83 (39 percent). Table 82 serves as an illustration of this.

From Tables 79, 80, 81 and 82 we see that almost four-fifths of the incorrect diagnoses come about in cases of diseases of the respiratory tract. We have obtained these data not only in 1941 but also in other years during the hospitalization of patients, young and older.

This is the kind of erroneous diagnosis of influenza made by physicians on sending the patients into the hospital. Similar errors in different seasons of the year and in different places have been noted in our material in 30-50 percent more cases.

Complications and Associated Diseases in Acute Catarrhs of the Respiratory Tract

During the period from January to April 1941 212 patients were admitted to our hospital with the following diagnoses: chronic diseases of the respiratory tract, 16 persons (7.5 percent); acute catarrhs of the respiratory tract, 113 persons (53.3 percent); and other diseases, 83 persons (39.2 percent).

Table 82

Various Diseases Erroneously Considered Influenza

Diagnosis	No. of patients	Percent[1]
Sinusitis (alone or in combination with chronic tonsillitis, sore throat, etc.)	33	39,8
Chronic rhinitis (alone or in combination with chronic tonsillitis, sore throat)	3	3,6
Follicular and lacunar tonsillitis (alone or in combination with chronic bronchitis etc,)	19	22,8
Pneumonia, pleurisy, bronchitis, pulmonary tuberculosis	10	12,1
Otitis	2	2,4
Malaria	10	12,1
Lymphadenitis, abscess of the hand, subpectoral abscess	3	3,6
Scarlet fever	1	1,2
Fever of undetermined origin	2	2,4
Total . . .	83	100,0

[1] The data are presented arbitrarily in percentages for purpose of convenience in comparison.

In looking over the composition of patients suffering from acute catarrhs of the respiratory tract, we directed attention to the high percentage of complications and associated diseases, chiefly of the respiratory tract. Among these patients there were a particularly large number of persons with chronic diseases of the respiratory tract, throat, nasal sinuses. Table 83 gives us a graphic idea of this.

As seen from Table 83, in 91 patients (out of 113) with acute catarrhs various complications or associated diseases were noted in the respiratory tract, etc. Only 22 persons with acute catarrhs of the respiratory tract did not have complications or associated diseases. They comprised 19.4 percent of the so-called pure cases of acute catarrh.

After a careful examination of patients by the laryngologist and the roentgenologist the percentage of "pure"

cases invariably decreased.

Table 83

Complications and Associated Diseases With Acute Catarrhs of the Respiratory Tract

Diagnosis	No. of patients	Percent
Pneumonia, pleurisy, pulmonary tuberculosis	16	17,6
Chronic bronchitis, laryngotracheitis, sometimes in combination with deviation of the nasal septum	10	11,0
Chronic sinusitis, sometimes in combination with chronic tonsillitis, sore throat, chronic rhinitis and others	38	41,8
Chronic tonsillitis, sometimes in combination with chronic rhinitis, bronchitis, otitis	23	25,3
Chronic rhinitis	1	1,0
Follicular tonsillitis	3	3,3
Total . . .	91	100,0

[1] The data are presented arbitrarily in percentages for purpose of convenience in comparison.

Naturally, the question arises: Are not the results of our observations in 1941 chance; were they not caused by the characteristics of the given year or local conditions (weather, specific working conditions, etc.)?

For the purpose of solving this problem let us turn to our clinical observations for 1939-1941 the results of which are presented on Table 84.

By complications and associated diseases we mean pathological changes of the respiratory organs as well as of the throat, ears and accessory nasal sinuses. These changes occur in influenza and in acute catarrhs and are produced by them or existed prior to them or accompanied them. We classify pneumonia, chronic bronchitis, sinusitis, tonsillitis, rhinitis, etc. among them.

From Table 84 it is seen that in patients with influenza and acute catarrhs in approximately the same month of 1939 and 1940 complications and associated chronic dis-

eases of the respiratory organs as well as of the throat and ears were noted. The highest percentage of these diseases (71) was noted among patients with acute catarrhs by comparison with patients suffering from influenza A (24) and influenza B (58). In 1941, during the period from January through April, that is, in the same months as in 1939 and 1940, we observed an even higher percentage of chronic diseases (83) among the patients with acute catarrhs. This was apparently produced by a certain predisposition of the latter to complications, is not of an accidental nature, and is explained by their increased sensitivity to chilling. Thus, 63.5 percent of the patients with acute catarrhs in 1941 associated their disease with a cold.

Table 84

Influenza and Acute Catarrhs of the Respiratory Tract with Their Complications and Associated Diseases

Diagnosis	Beginning of 1939		Mar. and Apr. 1940		From Oct. through Dec. 1939 and up to March 1940		From Jan. through Apr. 1941	
	No. of pts.	%	No. of pts.	%	No. of pts.	%	No. of pts.	%
Influenza A	108							
No complications of associated diseases	82	76						
With complications and associated diseases	26	24						
Influenza B			127					
No complications or associated diseases			53	42				
With complications and associated diseases			74	58				
Acute Catarrhs of the Respiratory Tract					205		129	
No complications or associated diseases					60	29	22	17
With complications and associated diseases					145	71	107	83

In the histories of more than two-thirds of the patients there was mention of previous "influenzas," sore throats, coryzas, repeated two or three or more times a year, and sometimes every month. More than eight percent

of these patients returned to our hospital during the course of the same year (one patient was in it three times).

Shteynshneyder and his co-workers (1934), examining 300 persons at a public health station, found that 83 percent of them suffered from chronic diseases of the respiratory organs, throat and nasal sinuses and only 17 percent were healthy.

Clinical observation made in 1944 showed that in 79 percent of persons suffering frequently from "influenza" and "acute catarrhs," chronic disease of the upper respiratory tract, throat, nasal sinuses was noted; in the others (21 percent) a chronic bronchitis with pulmonary emphysema, tuberculosis, or bronchiectasis were found.

From what has been stated it fo lows that among the patients with acute catarrhs very many suffer from chronic diseases of the respiratory tract, particularly of the upper respiratory tract as well as of the throat, ears and accessory nasal sinuses, which are frequently exacerbated. These exacerbations, not uncommonly taken from acute diseases and from time to time not readily distinguishable from them, are expressed in a deterioration of the local catarrhal signs and a general reaction of the body (fever, headache, etc.). They occur under the influence of "the cold" factor in persons sensitive to chilling.

The observations of laryngologists (Taytovich, 1926; Trutnev, 1936, and others) are interesting concerning the influence of pathological changes in the upper respiratory tract on various organs, for example, of antral sinusitis on the meninges (Trutnev), central and peripheral nervous system. Nasal respiration can hold back up to 90 percent of the microbes inhaled by man and, by the same token, prevent their penetration into the more distal respiratory passages. In the light of the observations of these otolaryngologists the data which we have obtained concerning the role of chronic pathology of the ENT organs in the occurrence and frequency of acute catarrhs of the respiratory tract acquire great importance. At the same time, it apparently has no notable effect on the influenza morbidity (Preobrazhenskiy, 1953; Popova, 1954; the data of Table 84).

Clinical Characterization of the Widespread Concept "Influenza" and "Acute Catarrhs of the Respiratory Tract". For the purpose of a better understanding of the clinical nature of influenza it is essential to make clear what constitutes the groups of diseases usually designated influenza and acute catarrhs of the respiratory tract. These diseases are not uncommonly confused with each other and frequently one is taken for the other. Among them the three following groups of diseases may be distinguished.

1. Influenza produced by the specific virus of the

A, A-prime, B, C, and D types.

2. Diseases similar to influenza which have been given the name of acute catarrhs of the respiratory tract or, according to the Instructions of the Ministry of Health USSR, "Acute Catarrhs of the Upper Respiratory Tract." The etiology of the majority of these diseases has not been clarified but in their origin great importance is ascribed to the chilling factor. Probably, a part of them are of bacterial origin, without the participation of the cold factor, particularly among children (Bychkova, 1951; Ritova, 1953, 1954; Zhdanov and Ritova, 1954); another part is produced by viruses (adenoviruses, common cold viruses and others), being, therefore, a complete group of different diseases.

At one time it was suggested that acute catarrhs be called "febrile, seasonal catarrhs". However, these terms are incorrect: the disease is not always accompanied by fever and is observed not only in certain seasons (cold, damp) but also the whole year round. We have previously suggested calling them "cold" diseases. However, this term is also inexact, because catarrhs of the respiratory tract of microbial and virus origin have been described which occur without any preceding chilling. The term "acute catarrhs" does not satisfy us, because many of them represent an exacerbation of a chronic process. However, for want of a better term we are still using this term. We prefer the term "catarrh of the respiratory tract" to "of the upper respiratory tract," because sometimes not only the upper respiratory tract but also the small bronchi and bronchioles are involved. Various diseases, erroneously considered to be influenza belong in this group.

Therefore, all the diseases which now figure in the common diagnoses of influenza and acute catarrhs of the respiratory tract can be divided into three groups and are included in the outline presented in Table 85.

1. Influenza produced by the specific virus type A, A-prime, B, C, D.

2. Catarrhs of the respiratory tract: a) acute and chronic catarrhs and excerbation of the latter occurring under the influence of the chilling factor; the etiology of them has not been clarified yet (apparently, of endogenous bacterial origin); b) acute catarrhs of bacterial origin (exogenous); c) acute catarrhs of virus origin.

3. Various diseases usually considered to be influenza. They are usually associated with a toxic state and catarrhal signs which accompany the disease and confuse the physician. Among the diagnostic errors sometimes diseases figure which are called influenza not because of their nature but rather on the strength of various formal consi-

derations (need for making a specific diagnosis, considerations of hospitalization, etc.).

Table 85

Outline of Diseases Considered to be Influenza

I. Influenza (epidemic, sporadic)

1. Types A, A-prime and A-double prime	2. Type B	3. Type C	4. Type D

II. Catarrhs of the Respiratory Tract

1. acute and chronic catarrhs of the respiratory tract and their exacerbations occuring under the influence of the chilling factor (endogenous bacterial origin)	2. acute catarrhs of bacterial (exogenous)	3. acute catarrhs of virus etiology (common cold viruses, adenoviruses)

III. Various Diseases Usually Considered to be Influenza

A. accompanied by catarrhal signs			B. not accompanied by catarrhal signs	
1. acute infectious diseases of different etiologies (primary atypical pneumonia, or nephrosis, measles, typhus and typhoid fever, croupous pneumonia, etc)	2. diseases of the respiratory tract of chemical and mechanical origin	3. catarrhs of allergic origin	4. infectious and non-infectious diseases in people with chronic disease of the respiratory tract, throat, nasal sinuses	5. various diseases accompanied by a toxic state taken for influenza

It should be emphasized that erroneous diagnoses of influenza not uncommonly occur as a result of a hasty examination of the patient and an inattentively taken history. In the majority of cases at the time of the first examination it is difficult to diagnose influenza and to exclude other diseases which have many features in common with it both according to the appearance of the patient and in the nature of the catarrhal or toxic manifestations.

At the current level of our knowledge catarrhs of the respiratory tract represent a generalizing concept of acute, chronic and also exacerbated chronic diseases. For purposes of making the typical diagnosis more exact it is important to distinguish whether we are dealing with an involvement of a single isolated organ, for example, the throat, nasal sinus, ear, etc., or several organs. The diagnosis should be formulated according to the anatomic name of the involved organ or a part of it, for example, rhimitis, antral sinusitis with an indication specifically of whether it is acute, chronic or exacerbated.

Simultaneous involvement of a number of organs of parts of them are also classified among the catarrhs with an indication of whether they are acute, chronic, or exacerbated forms. In order to clarify the localization of a pathological process these affected areas should be named, for example, "acute rhino-pharyngo-tracheitis," etc.

Clarity in defining the concept of "influenza" and in the correct diagnosis of the diseases covered by this term is not only of importance in principle but it is also of tremendous practical significance in respect to success of therapeutic measures proposed for the treatment of them.

61. Clinical Characterization of Influenza. General Symptoms

Influenza is an acute infectious disease which is distinguished by its high degree of infectivity and is accompanied by general toxic and catarrhal signs. The toxic state usually is manifested beginning with the very start of the disease; the catarrhal lesions of the mucous membrane of the respiratory tract may occur after a delay or may be absent. The incubation period in influenza is very short: from several hours to one or two days, rarely longer. Most often, influenza begins suddenly, without prodromal signs, which distinguishes influenza from a number of other infectious diseases. The general toxic state is expressed in a chill, headaches, sometimes dizziness, fever, depression and rheumatic type pains, as well as adynamia, hypotension, nausea, vomiting once or repeatedly, tendency towards perspiration, cutting pains in the eyes, etc. Along with the gen-

eral toxic signs a facial hyperemia develops, with congestion of the conjunctivae, sometimes of the sclerae, lacrimation, and photophobia. The condition of many patients appears extremely severe. Thereby, the severity of the clinical picture increases very rapidly, sometimes during the course of several hours, which distinguishes influenza from certain diseases which develop gradually (typhus and typhoid fevers, etc.). The patients are sluggish, apathetic, drowsy, sometimes somewhat excited, and suffer from insomnia. This acute, severe condition lasts usually for a short time, one to two to three days, and rarely four or five days if the cause of the disease does not have any serious complications. After this, a considerable improvement occurs in the patient's condition, and he recovers during the course of several days. Of the general toxic signs adynamia lasts longest, and this may make itself felt for quite a long time (one or two weeks or more) after the disappearance of all theother pathological signs.

Sometimes from the very beginning of the disease and more often after a certain delay catarrhal signs occur, local lesions appear in the mucous membranes of the respiratory tract. They are expressed in a scratchiness and raspiness in the throat, pains on swallowing, hyperemia and edema of the nasal mucosa, stuffiness of the nose, coryza, hoarseness and cough, frequently short, dry, barking, and sometimes with the excretion of a small amount of sputum. Coryza may at times be accompanied by considerable liquid serous or seromucoid excretions or by a thicker mucopurulent discharge. Sometimes, sneezing is observed. In certain patients there is nasal bleeding. The soft palate, fauces, uvula, tonsils, posterior wall of the pharynx are considerably injected. (The "granular appearance" of the soft palate ("military small vesicles on the soft palate" -- Rozenberg) which has been described by certain authors (Rozenberg, 1934; Morozkia, 1955 and others) as a sign pathognomonic of influenza has been observed by us not only in influenza but also in acute catarrhs of the respiratory tract, tonsillitis, Botkin's disease [infectious hepatitis] and others.) Sometimes the patient may note signs of laryngitis, laryngotracheitis, and bronchitis. However, all these local catarrhal signs become very quiescent (particularly at the beginning) before the general toxic state which accompanies influenza.

We consider it fitting to give a clinical characterization of influenza on the basis of the observations which we have made, without presenting numerous descriptions of it which exist in the foreign and Soviet literature (Glinchikov, 1922; Rozenberg, 1934; Rossiyskiy, 1945; Tushinskiy, 1944, 1953, and others; Leichtenstern, 1896; Marquie, 1898;

Hesse, 1918, Hoppe-Seyler, 1919; Schmensky, 1919; Spengler, 1919; Sen, 1923; Franke, 1930; Hegler, 1934; Frentzel-Beyme, 1950; Karpinski, 1953, and many others).

We should like to dwell on an analysis of the various manifestations of the toxic state.

Chill. The chill is not always marked. Most often, the disease begins only with a chilly feeling, and sometimes the chill is absent entirely. Not uncommonly, the patients note a chilly feeling on the second day of the disease, and from time to time, on the third day.

Dizziness. As an expression of the toxic state dizziness occurs beginning with the first few days of the disease. It can occur also when the patient is lying down. Not uncommonly, a brief period of dizziness observed in the later periods of the disease (third to fourth day) occurs if there is any violation of the bed-rest routine. In such cases, the dizziness passes quickly and is not the result of the toxic state but rather of the stay in bed for several days.

Headache. Headache may be very severe and is usually concentrated in the anterior half of the head -- in the supraorbital area, in the temples, in the forehead, which distinguishes influenza from a number of other infectious diseases in which the pains are felt throughout the head. The patients rarely complain of pains in the parietal area, in the occiput or in the entire head. Headache lasts for a short time and usually disappears when the temperature drops.

Fever. Certain infectious diseases are distinguished by a very typical temperature reaction, for example, malaria or relapsing and typhoid fevers. May this be said also with respect to influenza? In the literature there are indications that in influenza no strict rules and regulations are observed in the temperature curve (Ionin, 1940, 1942; Rossiyskiy, 1942, 1945, 1949, and others). However, this is not entirely true. Certain characteristics distinguish the temperature reaction in influenza. First, the temperature rise occurs quickly in the patients. As early as the first day of the disease the temperature reaches its maximum in the uncomplicated cases of influenza. If the temperature should rise on the next day it is only by tenths of a degree. This characteristic feature of the temperature curve distinguishes influenza from a number of other diseases (typhoid, typhus, etc.). Secondly, the fever in influenza is very brief. In from one to twelve percent of the cases (in different outbreaks) it lasts only one day; in 10-25 percent, two days; in 13-28 percent, three days; and in 20 percent, four days. According to our data, the temperature reaction lasts up to three days in 30-58 per-

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cent in uncomplicated influenza. The temperature remains longer than five days (maximum of six) as a rare exception. According to the data of Rozenberg (1931, 1934), the temperature lasted a total of one day in eight percent of the patients; two days, in 42 percent; three days in 26 percent; four days in 10 percent, that is, in 86 percent of the patients with influenza the temperature reaction lasted no longer than four days. If the temperature drags on we are dealing with complicated influenza. There is no uncomplicated "lingering" influenza. If the temperature does not drop to normal on the fourth day in the influenza patient (particularly on the fifth day) of the disease it is essential to look for some complication which might explain the presence of such a prolonged temperature. With respect to the diurnal fluctuations in the temperature, they sometimes do not occur, and after two or three days the temperature becomes normal. Frequently, a decreasing fever curve is observed, and from time to time, intermittent. In approximately 25 percent of the cases the temperature drops critically; usually it decreases gradually, and in the course of one or two days it reaches normal.

In approximately 1/4 of all cases the disease occurs with a low grade fever, and in 20-30 percent, with a temperature over 39°.

In the literature, mention is frequently made of the double-hump temperature curve which is, as it were, characteristic of influenza (see, for example, Jagic, 1918). Actually, in 7 to 25 percent of the patients a biphasic, or double-hump temperature curve is observed. However, we have managed to find it in the hospital only in complicated cases. The second hump of the curve is evidence only of an incipient complication (pneumonia, sinusitis, bronchitis, etc.), and exacerbation of a chronic disease (cholecystitis, pyelitis and others) or of increasing catarrhal signs under the influence of the microbial flora (purulent rhinitis and others). The authors, in indicating the double-hump temperature curve apparently did not analyze it properly and did not note the complications and exacerbations of chronic diseases which explain the second temperature rise.

We were unable to find any cases of influenza with a lingering temperature of two-three-days duration without any complications, and we doubt that such cases exist. There is either an error in the diagnosis (for example, a diagnosis of "lingering influenza" in typhoid with a negative agglutination reaction) or unrecognized influenza complications. We have had the opportunity to encounter such signs repeatedly.

Therefore, a distinctive feature of the influenzal fever is the rapid maximum development of the temperature

usually on the first day and the brevity of its course. We are presenting several temperature curves in influenza which we have observed during an influenza A-prime outbreak in 1952 (Fig. 175).

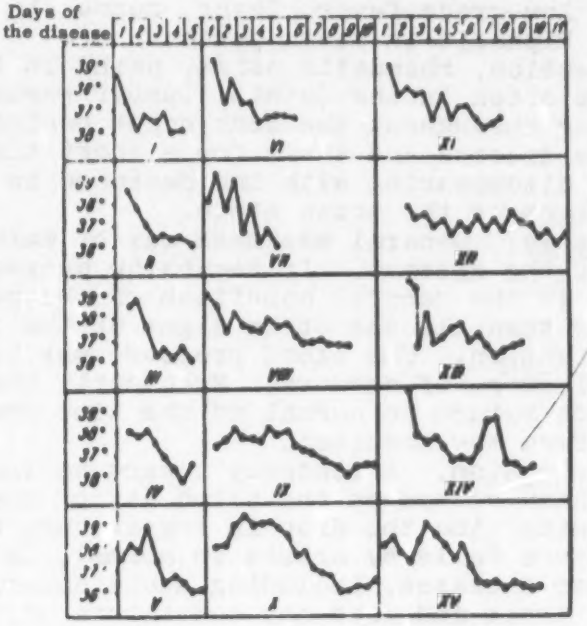

Fig. 175. Temperature Curves in Influenza

In 3.5 percent of the patients, according to the observations of a co-worker in our hospital, Shimshelevich, during an influenza A-prime outbreak in 1949 and based on outpatient material the disease had a course with toxic signs but without a temperature reaction. The diagnosis of influenza was confirmed not only epidemiologically and clinically but also in the laboratory, including serologically.

Certain authors (Leichtenstern, 1896) doubt the existence of afebrile forms of influenza and feel that in these cases a fever which lasted only a short time went unnoticed. Nevertheless, Stuart-Harris and co-authors (1938) indicate that in 17 percent of influenza patients there was no temperature reaction (this percentage is higher than that mentioned by Shimshelevich).

If we examine the nature of the temperature curves in cases of uncomplicated influenza the following types may

be distinguished among them:

I. Fever and critical drop in it in 23 percent.
II. Fever, low-grade fever, normal in 62 percent.
III. Low-grade fever, afterwards normal in four percent.
IV. Low-grade fever, fever, normal in four percent.
V. Biphasic in seven percent.

Depression, rhemuatic pains, pains in the bones, muscle, less often in the joints, lumbar region, extremities, back or throughout the body occur beginning with the onset of the disease and lasts for a short time, one-two-three days, disappearing with the decrease in the fever and the other signs of the toxic state.

Adynamia. General weakness may be marked at the beginning of the disease. It begins to decrease with the improvement in the general condition of the patient and lasts longer than all the other signs of the toxic state.

Hypotension. The blood pressure may be lower than 100/50 millimeters of mercury. Frequently the blood pressure does not return to normal at the time the patient is discharged from the hospital.

Perspiration. A tendency toward an increased perspir tion which occurs on the third day of the disease and later, at which time the drop in temperature begins or when the temperature falls by crisis to normal, is observed also in many other diseases, including acute catarrhs of the respiratory tract and does not constitute anything characteristic of influenza.

Eye Symptoms. Eye symptoms appear very early in the form of a sensation of cutting, pains in the eyeballs, particularly on moving the eyes or on compression of the eyeballs as well as hyperemia of the conjuctivae, sclerae (less often), sometimes in the form of lacrimation and photophobia. Changes in the eyes in combination with hyperemia and puffiness of the face give the influenza patient a characteristic appearance which distinguishes him from patients with other diseases, for example, acute catarrh of the respiratory tract. With the decrease in temperature and lessening of the intoxication the eye symptoms gradually disappear.

Nausea and single or repeated vomiting as well as other signs of a functional involvement of the nervous, vascular system, etc. also observed from time to time at the beginning of the disease belong to the signs of the toxic state. Vomiting can sometimes be associated with the taking of drugs, for example sulfonamides.

62. Changes in the Organs and Systems. Skin

The increased tendency of the patients toward perspiration is striking from the very start of the disease. The absence of dryness of the skin and sometimes moisture of it in the presence of quite a high fever should attract considerable attention on the part of the physician in differentiating influenza from the typhoid type of disease, particularly from typhus and relapsing fever.

As far as the rash in influenza is concerned which has been described by various authors (Glinchikov, 1922; Rabinovich, 1927; Sadovskiy, 1928; Komarova and Barysheva, 1929; Braude and Freyfeld, 1930; Veynberg-Niklas, 1931; Derbandiker, 1931; Gorchakov, 1936; Krivovyaz, 1937, 1938; Pirlik, 1938; Fedotov, 1939, 1945, 1948; Ionin, 1940; Rozen'yer and Sizemova, 1948 and others), at the present time these data should be considered erroneous. Apparently, the old authors were right (Filatov, 1885 and Leichtenstern, 1896, as well as Koltypin, 1923 and Zyukov, 1949), who denied the existence of a rash in influenza with the exception of herpes and sometimes a heat rash.

In the analysis of the works of the authors mentioned above and of others (like, for example, Shteynberg, 1892; Sokolov, 1919; Hainiss, 1919; Jacob, 1919; Leimdörfer, 1920; Walter, 1929) it is easy to show that in all these cases diagnostic errors occurred, and sometimes gross ones (Rabinovich, 1927), which are explained by the absence of any firm knowledge of the etiology of influenza at that time.

Although Korovin (1939, 1941) mentioned that herpes is less characteristic of influenza than of acute catarrh of the respiratory tract, we have observed it in 10-25 percent of influenza patients in various epidemics, that is, no less often than in the acute catarrhs. Rozenberg (1934) considered the frequent eruption of herpes typical of influenza.

To be sure, in influenza incidental skin eruptions can occur (furuncles, acne, lichen, eczema, epidermophytosis, or a pustular rash); however, they do not have any pathogenetic connection with it.

During the pandemic of 1918-1919 and in various other outbreaks we have not observed any rashes in influenza.

In the interest of making the correct diagnosis in various acute diseases accompanied by a rash influenza should be considered last, and another source of these eruptions should be sought.

63. Respiratory Organs

In influenza the respiratory apparatus is most frequently involved. The involvement of the lungs is the most serious complication of influenza, and leads to a fatal outcome more often than the other complications.

The respiratory organs, particularly the upper respiratory tract, represent the place where the various pathological processes unfold in various sections and sometimes throughout their entire extent. Influenza is usually accompanied by rhinitides, pharyngitides, tonsillitides, nasopharyngitides, laryngitides, tracheitides, bronchitides and sometimes bronchiolitides and pneumonias of different forms and duration. In certain cases the pathological process extends to adjacent organs directly connected with the respiratory tract. Involvement of the nasopharynx, throat, accessory nasal sinuses (central sinusitides, frontal sinusitides, ethmoid sinusitides and sphenoid sinusitides), of the auditory apparatus (inflammations of the eustachian tube, otitides of different kinds) of the eye -- tear ducts (dacryocystitides) are observed in addition to frequent involvements of the conjuctiva of the eye.

In addition to headache, depression, etc. the patients frequently complain of difficult nasal respiration, pains in the larynx, sometimes in the windpipe, behind the sternum or in the entire chest, cough, which afterwards may be accompanied by the excretion of various quantities of sputum of different character (mucous, mucopurulent, bloody). Frequent complaints are hoarseness and pains ("shooting") in the ear. The subjective complaints may be accompanied by an inflammation of the nasal mucosa with the appearance of exudates of different natures and consistencies: liquid, acqueous, transparent and serous of thicker, less transparent mucous or mucopurulent. Nasal hemorrhages are observed (in our material in 2-14 percent in various epidemics), more frequently in persons in whom they existed prior to the case of influenza. Stuart-Harris and coauthors (1938) observed nasal hemorrhages in 15 percent of influenza patients.

In influenza an involvement of the accessory nasal sinuses is noted (sinusitides), sometimes with an extention of thepathological process to all of its sinuses (pansinusitis). The nature of involvement of the nasal sinuses may be different, beginning with catarrhal and ending with purulent (Engel'man, 1952; Radzimirskiy, 1952). The frequency of sinusitis varies in different influenza outbreaks. The involvement of the upper respiratory tract is usually of the nature of rhinitis, laryngitis, etc., being accompanied by hyperemia, swelling of the mucous membrane, sometimes,

marked with small hemorrhages, diphtheritic membranes, and bloody or purulent mucus (Davydovskiy, Dvizhkov and Chalisov, 1938). Sometimes, they assume a more severe nature in the form of a local swelling or considerable laryngeal edema or even tissue necrosis and croup (A. G. Filatov, 1928; Yuzefovich, 1937; Koltypin, 1924; Davydovskiy, 1938, 1939).

On rhinolaryngoscopy changes may be found in the nasal mucosa of different types and degrees (swelling, hyperemia, tumescence, particularly of the middle conchae), hyperemia of the tonsils, fances, uvula, soft palate, posterior wall of the pharynx, more or less pronounced throughout or only in one part. Koldobskiy (1951, 1953) observed dryness of the mucous membranes of the nose and pharynx in almost all influenza patients at the beginning of the disease, moderate congestion and swelling of the inferior conchae, sometimes a bleeding mucous membrane. The mucosa of the pharynx is slightly hyperemic during the first few days of the disease, and in the majority of cases it is moderately dry, sometimes with a violet hue in the area of the soft palate and uvula. Not uncommonly, during the course of the disease the lymphoid structures of the pharynx are involved (influenzal catarrhal angina).

In the work of Undrits and Koldobskiy (1953) it is mentioned that, in contrast to acute catarrh of the respiratory tract, the characteristic feature of influenza is a marked dryness of the nose (in 82 percent of the patients) on the first to fourth day of the disease. In presenting a description of the mucous membranes of the upper respiratory tract they direct attention to the violet hue of the soft palate and uvula.

Fotin (1954) indicates cyanosis of the conchae in influenza; sometimes, the cyanosis spreads to the floor of the nasal cavity and to the septum, and from time to time also to the soft palate and posterior wall of the pharynx (in a less pronounced form). The author considers a symmetrical cyanosis of the mucous membrane a symptom specific for influenza (he observed it in 34 out of 40 patients with influenza). Ostapkovich (1953) presents approximately the same picture. Sometimes localized, unilateral changes in the pharynx are found which are difficult to distinguish from chronic processes, for example, from tuberculous processes (A. G. Filatov, 1929). The laryngeal involvements, according to Koldobskiy, are encountered less often than the nasal, nasopharyngeal and pharyngeal involvement, whereas acute influenzal bullous laryngitides with a transition of the inflammation to the cords and with marked hoarseness are noted only in solitary patients. Finely punctate hemorrhages are also observed on

the laryngeal mucosa.

The mucous membrane of the trachea and bronchi is inflamed, markedly hyperemic. The sputum excreted in influenzal bronchitis may be no different from the sputum in ordinary bronchitides, but sometimes contains an admixture of blood.

The influenzal disease of the bronchi is of a variable extent. The process may be limited only to the large bronchi, may involve the medium-sized bronchi, and less often it goes deeper along the bronchial branches to the small bronchi and bronchioles. It may be localized, involving one lobe of the lung, but sometimes it involves all the lobes or some of them. There are also indications that signs of asthma occur in influenza (Marques, 1925). The involvement may pass on to the pulmonary parenchyma, producing pneumonias of different types, of various localizations and of different extents (Sokolov, 1889; Magat, 1919; Etinger, 1924; Becher, 1918; Mandels, 1933; Milash, 1941). They can be of an acinar or military form (Uspenskiy, 1933; Gel'fi and Sigachev, 1936; Abramova, 1937; Davydovskiy, 1939; Rissoto, 1934, etc.). The process may gradually shift to adjacent areas -- creeping pneumonia (Ornatskiy, 1936). Individual areas may become confluent with one another, forming lobular pneumonias. These foci may be solitary or multiple, scattered throughout one or several lobes of the lungs (Brumshteyn, 1936), giving a variegated picture on physical examination of the patient; the areas where there is a clear pulmonary percussion sound are encountered alternately with dullness, and sometimes alongside areas where the percussion sound is amphoric. The same variegated picture may be obtained also on ausculation of the lungs (Shervinskiy, 1918; Faynshmidt, 1919; Konchalovskiy, 1927; Chistovich, 1938; Blyum and Erez, 1940; Shklyar, 1946; Zislin and others, 1950).

A no less variegated pathological picture is observed on autopsy. The pneumonias may be hilar (Levenshteyn and Gutman, 1937), central (Uspenskiy, 1933) as well as cortical and peripheral (D'yachenko, 1946). They may be of the nature of an acute hemorrhagic edema (Svenson, 1936, etc.).

Pneumonic lobular foci which are confluent with one another may fill an entire lobe or may resemble a picture of lobar pneumonia. Actually, these are not lobar but rather pseudolobar pneumonias consisting of various lobular foci with a variegated picture on percussion and auscultation depending on the stage of development of the pneumonic foci in various lubules of the lungs, which also distinguishes them from croupous inflammation of the lungs. In the pseudolobar pneumonias rusty sputum is usually not

observed, and bronchial breathing is usually not heard. Such pneumonias are distinguished from croupous pneumonias by the absence of leukocytosis, which is explained by the depressive influence of the influenza virus on leukopoiesis.

At autopsy various sections of the lungs are well seen, affected by pneumonia at various stages of its development (Abrikosev, 1941; Davydovskiy, 1938, 1939).

Glinchikov (1922, 1938) describes pneumonias of the Grancher type in influenza or splenopneumonia. Clinically, an area of uninterrupted dullness, marked weakening or even complete loss of the respiratory sound and of vocal fremitus in the area of the dullness is noted. These pneumonias present definite diagnostic difficulties in distinguishing them from exudative pleurisies. Grancher observed them in the lower lobes of the lungs; Glinchikov, in the upper lobes where they imitated encapsulated pleurisies.

Kogan (1944) described acute interstitial (atypical) pneumonias during an outbreak of influenza in December 1943 and in January 1944. The author did not succeed in establishing whether or not these were influenzal (virus) pneumonias, pneumonias of bacterial origin complicating influenza, or so-called "primary atypical pneumonias". Similar descriptions have been given by Brethauer and Thompson, 1944. The problem of the nature of interstitial pneumonias in influenza has not been decided yet.

The pneumonias described above by various authors are apparently secondary complications of influenza and are caused by the microbial flora (pneumo- and streptococci, Bacillus influenzae). Influenzal pneumonias are also observed which are accompanied by the excretion of a liquid serosanguineous sputum. Pneumonias of hemorrhagic nature were encountered particularly frequently during the pandemic of 1918-1919 (pneumonias of the "Spanish Flu" type). Beginning with 1939 we noted four cases of such pneumonias: in December 1933 during an outbreak of influenza A; in January 1947 during an outbreak of influenza B; in January and March 1953 (Epshteyn and Levinson, Epshteyn and others, 1949-1950; Knyazeva and Zayrat'yants, 1954). These and primary (virol, hemorrhagic) influenzal pneumonias occurring during the first few days of the disease (early pneumonias) which occur in an extremely severe form, with a marked toxic state, dyspnea, cyanosis, tachycardia, and which rapidly lead to a fatal outcome.

If they last for several days the pneumonias are accompanied by the expectoration of a mucosanguineous sputum. However, sometimes they culminate fatally on the first or second day after the onset of the disease, at which time there can be no sputum. The diagnosis of them is made on the basis of the pronounced toxic state and the general se-

vere course of the pneumonia. On the examination of these patients the most varied picture is found in the lungs depending on the number of pneumonic foci, their massiveness and distribution. In 1933, we observed the picture of lobar pneumonia with bronchial breathing and other signs in in the left lung of one patient. On the first few days of the disease the patient expectorated a mucopurulent sputum, which afterwards became mucosanguineous and remained so until thepatient's death, which occurred on the fifth day after the beginning of the disease.

At autopsy, a confluent fibrinous pneumonia was noted in the upper lobe and a round-focus pneumonia of the lower lobe of the left lung with multiple foci of hemorrhages in them. In addition, a small-focus hemorrhagic pneumonia was found in the right lung.

In a patient whom we examined in 1947 there was absolutely no sputum expectorated. Auscultation and percussion of the lungs showed a variegated picture. The patient's condition was extremely serious: cyanosis, dyspnea (superficial respiration of 60 per minute with the participation of the accessory respiratory muscles); the pulse was 132 a minute and weak. The patient was admitted to the hospital on the fourth day after the beginning of the disease and on the first day of the marked deterioration in her condition, which occurred in connection with the onset of pneumonia. She was in the hospital for less than a day. Death occurred on the fifth day of the course of influenza and on the second day of pneumonia. The fact that the pneumonia was of this duration was determined also by the prosector who performed the autopsy. At autopsy an acute catarrhal-hemorrhagic laryngotracheobronchitis and a bilateral large-focus catarrhal-hemorrhagic and fibrous-hemorrhagic pneumonia were found. The microbiological examination was negative. The influenza B virus antigen was found; however, the strain of the influenza virus could not be isolated.

The fact that these hemorrhagic pneumonias come from the influenza virus was confirmed in the investigations of Romanenko (1953) during an outbreak of influenza in 1946, at which time he was able to isolate two A virus strains from sections of hemorrhagic pneumonias. Afterwards, he repeatedly isolated influenza virus strains from foci of hemorrhagic pneumonias in the fulminant forms of influenza or noted the presence of influenza virus antigens in the hemorrhagic pneumonic foci in persons who later died and came to autopsy both during outbreaks or increases in the morbidity rate of influenza and during the interepidemic period. Influenza type A and B virus strains were also isolated from hemorrhagic pneumonias by foreign authors (Himmelweit, 1943), Finland and others (1945). Kaganskaya (1952)

writes about the viral nature of early pneumonias in influenza.

As far as the secondary pneumonias are concerned, the pulmonary complications of influenza which are of microbial origin, they can develop early, as early as in the first few days of the disease when the influenza is at its climax. They can occur towards the end of the course of influenza, sometimes during the period of convalescence. The severity of their course depends on a number of conditions: the nature of the pneumonias, the pathogens, but chiefly on the condition of the patient himself. The earlier the pulmonary complication occurs in influenza the more severe its course, because it represents an additional microbial infection superimposed on the main virus infection.

When the pneumonia occurs during the period when the body is free of the influenzal toxic state it has a more benign course, because the organism can cope more readily with this new additional infection. This has been noted also by Tushinskiy, Korovin and Kovalev (1946). Pneumonias which develop at the beginning of influenza can proceed with a normal leukocyte count or even with leukopenia.

Secondary pneumonias are accompanied by leukocytosis (usually slight) when they occur at the end of the influenza or during the period of convalescence, at which time the depressive effect of the influenza virus on leukopoiesis is reduced or stopped.

We do not agree with Tushinskiy and Korovin (Tushinskiy 1944, 1945, 1946, 1951; Tushinskiy, Korovin and Kovalev, 1946; Tushinskiy and Korovin, 1949; Korovin and others, 1953), who believe that the early pneumonias are virus pneumonias; those which occur later, on the third to fifth day of influenza, are virus-bacterial; the pneumonias which occur still later, after a week or more, are bacterial. Nor can we understand why Lichtenstein (1952) considers that eight percent of the influenza complications which he noted in 1949 were pneumonias of virus-bacterial origin, although in the sputum of these patients pneumococci, diplococci and streptococci were found. The authors do not present any convincing proof on behalf of their conception, which, incidentally, sounds somewhat mechanistic.

The final solution to the problem of the etiology of the early pneumonias in influenza can be reached only after the appropriate microbiological (virological, bacteriological) investigations.

The duration of the pneumonias is different. Sometimes, they are eliminated exceptionally quickly, and in several days no traces remain of them, particularly in the localized small lobular foci. More often, they are gradually absorbed and sometimes are of a serpiginous nature (par-

ticularly in the lower lobes of the lungs) and they assume a lingering, sluggish course which terminates in pneumosclerosis [pulmonary fibrosis]. From time to time, these pneumonias may drag on for two or three months or longer, even years (Gel'fer and Sigachev, 1936; Svenson, 1937; Abramovich, 1940). Rappoport (1933) calls them "pneumonia lenta." They can be accompanied by a low-grade fever and by the symptoms characteristic of pneumonia. Sometimes, there is no dullness in the lungs. Such lingering pneumonias give us the grounds for confusing them with tuberculosis. The X-ray picture and the absence of tubercle bacilli in the sputum assist in making the diagnosis.

We have had an opportunity to observe lingering pneumonias, chiefly in older persons suffering from pulmonary fibrosis or pulmonary emphysema, who have become sick in the autumn or winter. These pneumonias frequently drag on until the spring.

In the literature dry pleurisies and catarrhs of the upper lobes of the lungs have been described wherein influenza facilli rather than tubercle bacilli have been isolated (Kindborg, 1926). Also, diaphragmatic and mediastinal pleurisies have been observed (Müller, 1921).

The pneumonias described above, representing a complication of influenza, can in their turn produce further complications in the form of suppurative processes (abscess, pulmonary gangrene), various forms of pleurisy and pulmonary fibrosis which remain after an unresolved pneumonia and which lead subsequently to the development of pulmonary fibrosis and bronchiectasis. In the literature it is possible to find mention of the fact that influenzal pneumonias have a severe course, that they frequently produce suppurative processes (abscess, pulmonary gangrene, bronchiectasis), in connection with which the prognosis in them is not uncommonly doubtful. We believe that this opinion was conceived by the authors on the basis of observations of the most serious cases of influenza complicated by pneumonia, because such patients usually are sent to the internal medical departments of the hospitals for treatment. In the material of our hospital (ordinary cases of influenza encountered every day), in which we were interested with the aim of studying the clinical picture of uncomplicated influenza, we had an opportunity of observing a large number of secondary influenzal pneumonias. We have rarely been able to observe a particularly severe course in a case of pneumonia. True, we used sulfonamides, penicillin and combinations of them extensively for treating the pneumonias; we usually noted the pneumonias when they first appeared. (We used not only clinical observations but also repeated laboratory and X-ray investigations with the slightest suspicion of pneu-

monia). The observations of the authors mentioned above pertained chiefly to the period before sulfonamides and antibiotics. Still another factor should be emphasized in our observation. Usually people in the prime of life came to our hospital; rarely, persons over 50 or particularly over 60 years of age. We had no cases in infants, particularly in sucklings nor in persons of advanced age, for whom pneumonia posses a great threat.

We have, at times, had the opportunity of noting the presence of a dry pleurisy, which usually disappeared quickly.

With respect to the pathogenesis of secondary pneumonias it is believed that they, apparently, are of bronchogenic origin, although cases of hematogenic and lymphogenic origin have not been excluded (Tushinskiy, Svenson, Rybakov, 1936; Brevdo, 1936). The respiratory lesions may occur par continuitatem as a result of the penetration of the infection from the nose and nasopharynx along the bronchial branches down to the alveolar tissue as well as to neighboring organs which are in communication with the respiratory tract -- the accessory nasal sinuses, the eustachian tube and the middle ear.

As has been mentioned above, secondary influenzal pneumonia is the result of a mixed additional infection, a superinfection. The influenza virus injures the epithelium of the mucous membrane of the respiratory tract (Smorodintsev, 1938; Malomuzh, 1950, 1952). It depresses the protective forces of the body and reduces the resistance of the mucous membranes when microbes act on them. Among the latter, the X-group of pneumococci, hemolytic streptococci, staphylococci, Bacillus influenzae, the Micrococcus catarrhalis, which increase considerably in numbers in the nasopharynx and pharynx during the first few days of the disease (by a thousand times according to Smorodintsev) are of principal importance. According to Zil'ber and Baydakova (1945), the influenza virus increases the permeability of the pulmonary epithelium.

Mention was made above concerning the virus nature of the primary hemorrhagic pneumonias.

It is very probable that the fulminating fatal pneumonias of the "Spanish Flu" type were of the same virus origin (1918-1919), although various bacteria were also isolated in them not uncommonly (Heath, 1919).

We should also like to mention the primary atypical (virus) pneumonias, which previously were erroneously taken to be influenza and only recently have been separated out as a special nosologic entity. Atypical pneumonias have been described by Kogan, Longcope, 1940; Rabukhin, 1945; D'yachenko, 1946; Skvortsov, 1946, 1947; Nikulin, 1947. It

has been established experimentally that the primary atypical pneumonias are not bacterial but rather are of virus origin (Dingle and others, 1943, 1945). They are caused by specific viruses, which it has not as yet been possible to isolate, rather than by the influenza virus.

The catarrhal signs in the respiratory mucosa in influenza may be delayed for 12-24-48 hours at the beginning of the disease, particularly if there are no chronic lesions in these membranes. Coryza and cough are not obligatory symptoms for influenza. Thus, for example, during outbreaks of influenza in 1952 they were absent in four percent of our patients, and in 1954, in 14 percent. Influenza without coryza or cough or other catarrhal signs has been observed quite frequently.

64. Cardiovascular System

There is no complete agreement in the description of the condition of the circulatory organs in influenza. This is related to the kind of material which has been subjected to analysis in the sense of severity of the course of the disease. In general, the pulse corresponds to the fever. Frequently, tachycardia is encountered, and there is also bradycardia. Arrhythmia, dicrotic pulse, acute cardiac weakness and acute dilatation of the heart are observed. The vascular permeability is increased (Grossman, 1919; Hottinger, 1925; Gottheil, 1929; Grünbaum, 1932; Ferraboue and Guichene, 1935; Egedy, 1934; Hamburger, 1938; Tregubova, 1949, and others).

Rossiyskiy (1942) mentions lability of the pulse, change in rhythm, extrasystoles as well as muffling of the heart sounds and the appearance of functional murmurs and cardiac dilatation in influenza.

There are also indications (Shtekhelin, 1936) that the cause of death in influenza is almost always cardiac weakness. However, endocarditis, myocarditis and pericarditis in the form of anatomic changes are extremely rarely encountered (Dressler and Kiss, 1929; Yerufeyev, 1936; Abdulayev, 1938). Sometimes, a functional weakness of the heart remains which may be expressed in palpitation, dyspnea on physical exertion, extrasystoles and unpleasant sensations in the heart area. As a rule, these signs disappear after several months.

According to Glinchikov (1948), the heart suffers in all forms of influenza, which is expressed in cyanosis, dilatation of the jugular veins, dyspnea and a change in the pulse. The heart dilates, its sounds become muffled, impure, and a systolic mumur is heard; myocardial degener-

ation is observed (in 50 percent of the cases), and from time to time endocarditis or pericarditis is encountered. The systolic blood pressure drops markedly (variations from 74 to 100; in individual cases, from 100 to 120); the diastolic pressure remains normal or even increases; the pulse pressure decreases. Abdulayev and co-authors (1938) did not find any reduction in the pulse pressure.

According to Konchalovskiy (1927), in influenza a functional involvement of the heart is frequently observed but there are also endo-, myo-, and pericardial lesions.

In influenza, according to the data of Abdulayev and co-authors (1938) the greatest drop in blood pressure was noted toward the end of the febrile period. According to the data of Stuart- arris and co-authors (1938) the greatest drop in blood pressure was noted toward the end of the febrile period. According to the data of Stuart-Harris and co-authors (1938), the blood pressure at the time of discharge in the patients from the hospital was lower than at the beginning of the disease by 15/10 mm. The average figure of the systolic blood pressure was equal to 95 mm.; in two-thirds of the cases the diastolic pressure was less than 50 mm. On recovery the blood pressure usually returned to normal after five to seven days, but in certain cases a further drop in it was observed with a return to normal only after 10-12 days. The authors observed cardiac insufficiency in only 0.5 percent of the cases.

Wal'dman (1926-1937), Abdulayev, Kadymov and Mamedova (1938), and Agalarov (1939) noted a decrease in venous pressure in influenza. The venous pressure, according to Kofman (1937), does not change much in uncomplicated influenza but it decreases when it is complicated by pneumonia. Phlebitides and thromboses of the veins and arteries in influenza have been described by Arsen'yev (1938), Nikulina (1938) and a number of other authors (Hottinger, 1925; Corten, 1930; Kornblit, 1935; Ferraboue and Guichene, 1935; Gruzin, 1940). The vascular system usually suffers in influenza, whereby this is sometimes extremely severe, bringing the patient to a state of collapse and a fatal outcome. This is understandable from the pathogenesis of influenza, which usually leads to an involvement of the blood vessels and a depression of the sympathetic functions of the vegetative nervous system as a result of the toxic influence of the vasomotor nerves. In Zakstel'skaya's work (1951, 1953) on the toxic effect of the influenza virus with respect to the capillaries this was shown well. Fortunately, the severe changes in the cardiovascular system associated with collapse are very rarely observed. However, they still all occur (Agroskin, 1928; Levina and others, 1948).

During the influenza outbreaks which we observed beginning with 1939 we did not find any kind of persistent or serious changes in the circulatory organs.

The cardiovascular changes were usually not marked, and they could be noted only by instrumental examination (Marut, 1934; Levina and co-authors, 1949, and others). Clinically, these changes could not always be observed. More frequently, they were expressed in lability of the pulse, sometimes in bradycardia as well as in tachycardia. The latter occurs more frequently in the influenza complications (pneumonias and others) and in patients with associated cardiac diseases. Much more often the pulse corresponds to the temperature. Nevertheless, sometimes a muffling of the heart sounds is noted and from time to time dilatation of the heart, the occurrence of a murmur or arrhythmia (7.5 percent of the cases in the 1946-1947 epidemic), usually of the extrasystole type. These signs disappear with the improvement of the patient's general condition. Bigeminy or trigeminy are exceptions. In 1947, we observed arrhythmia of the trigeminy t pe in a patient with influenza during the first few days of the disease, and this was followed by extrasystoles, and finally by normal rhythm. At the climax of the disease the toxic state produced the arrhythmias, and the latter disappeared simultaneously with them.

In 1947, during an outbreak of influenza B we found a dicrotic pulse at the climax of the disease, which lasted several days and then disappeared, in almost one-tenth of our patients (chiefly in young patients and adolescents). Before the 1946-1947 outbreak we were not able to observe this phenomenon, although certain authors mention it (Rozenberg, 1934, etc.). The muffled heart sounds or a slight systolic murmur are sometimes heard in the patients; these disappear during convalescence. The heart borders usually remain within normal limits.

As a rule, arterial hypotension is observed, sometimes quite considerable (systolic pressure less than 100-90 mm.; diastolic pressure less than 60-50 mm.). According to our observations, in 1934, 1944 the hypotension not only did not disappear on recovery of the patient, but, on the other hand, even increased. A simultaneous reduction in both the systolic and the diastolic pressures was observed (from 5 to 20 mm. of mercury), and sometimes in just one of them. We did not observe any marked hypotension. The blood pressure was lower than 95-90 mm. (systolic) and 55-50 mm. (diastolic) as an exception. We did not find any particular change in the pulse pressure. Probably, the hypotension comes from various causes. It is associated with hypadrenalinemia, with depression of the sympathetic

and increase in the parasympathetic functions of the vegetative nervous system with involvement of the vasomotor center and, perhaps, with a certain weakening of the cardiac muscle.

Other data on the blood pressure coincide with the observations made by Vasil'yev (1937), Abdulayev (1940), Belyayeva (1949) and others.

Investigations of the cardiovascular system carried out by Belyayeva in the influenza hospital in 1949 during an outbreak of A-prime influenza showed that in influenza there is a decrease in the venous pressure and a slowing in the circulation time. On capillaroscopy a dilatation of the capillaries, turbidity of the background and sometimes the formation of capillary aneurysms are found. On electrocardiography a lengthening is found in the P-Q interval and a flattening of the T wave. These usually do not return to normal at the time the patients are discharged from the hospital. Similar statements are encountered in the literature also (Wel'dman, Agalarov and co-workers, Abdulayev and co-workers, Ar'yeva (1951), Sokolinskiy and co-authors (1952) and others.

We have not had the opportunity even once of observing marked changes in a cardiovascular system which ended in catastrophe or led to serious circulatory disorders associated with serious consequences for the patient.

The stethoscopic cardiac changes which have been mentioned usually disappeared at the time of convalescence.

The Konchalovskiy sign (tourniquet sign) was found to be negative in influenza patients where there was no particular cause for it, for example, the presence of essential thrombocytopenia.

Digestive Organs. Many authors have not found any specific changes characteristic of influenza in the digestive organs. Others, for example, Nikulina, Glinchikov, Koltypin, Lorovitskiy and Medvedev (1927), Gutner (1927), Sandler (1928), Davydov (1929), Kushev (1932), Prokof'yev (1936), Gal'pern (1937), Cherkasskiy (1937), Müller (1921), Mendershausen and Kohn (1926), Edelmann (1928), Seeman (1928), Abels (1928), Lecaplain (1929), Wachsmuth (1929), Plaschkes (1931), Wall and Wetzel (1931), Taterka (1931), Goldi (1933), Winternitz (1934), Hudacaek (1938), Übermuth (1938), Calvy (1948), and Knothe (1950) observed considerable changes in the gastrointestinal tract in a number of cases, for example, a dry tongue, sometimes very much coated with a dirty membrane, a feeling of weight in the epigastrium, bloating of the abdomen, sometimes vomiting, loss of appetite, increased thirst, dryness in the mouth, liquid stool up to 10-15 times a day, sometimes teneemus, slight tenderness throughout the abdomen, and sometimes mucus and

blood in the stool (Glinchikov).

These changes gave the authors the grounds for speaking of different forms of influenza gastrointestinal, intestinal and abdominal (Alexander, 1921). We should like to add that not only during the pandemics but also during the epidemics and interepidemic periods many of the cases of the so-called intestinal forms of influenza should be classified in the typhoid-like, dysentery and other acute gastrointestinal disease groups (gastritis, enteritis, colitis, gastroenterocolitis, and others), which we have observed repeatedly.

If we lay aside the various quite rare casuistic cases of serious complications in the digestive organs described in the literature it may be stated that in influenza (and not always) dyspeptic signs, common to all febrile diseases, are observed: loss of appetite, coated tongue, constipation; from time to time, nausea, vomiting, and abdominal pains. This has also been noted by authors who present descriptions of the clinical picture of influenza. As an example we should like to point to the data of Nechayev and co-authors (1938) pertaining to the influenza outbreak in 1938. In the majority of cases only a loss of appetite and constipation for two or three days were observed. The gastric juice which was investigated a day after the fall in temperature in 30 patients was found to be normal in 12 (40 percent), of increased acidity in five (17 percent), and of decreased acidity in 13 (43 percent). However, we do not know what the gastric function was before the patient developed influenza. Of 48 persons who had previously suffered from gastridites and cholecystitides exacerbations of these diseases were observed in 20.

The diagnosis of influenza should be made on an objective, laboratory (biological or serological) basis in order to be convincing. Many diseases described by Alexander (1921) as the intestinal form of influenza were apparently a rather acute dysentery. Quite a few cases of acute and exacerbated chronic dysentery as well as other gastrointestinal diseases have been described as abdominal or intestinal forms of influenza. Bosse (1935) notes that among 22 of his patients (children) with the intestinal form of influenza 12 had had colitis in the summer-time shortly before the occurrence of influenza, and the others had paratyphoid or dysentery in their histories.

As far as our own observations are concerned we have, as a rule, not observed any particular changes in the digestive tract in influenza. Nausea and vomiting observed in the patients are usually of cerebral or toxic origin and are sometimes related with the taking of sulfonamides.

There was no heartburn or belching noted. Once we had an opportunity of observing hiccoughing in a patient from the very beginning of the disease. With the lessening of the toxic state the hiccoughing stopped and reappeared in this patient, at which time a complication occurred -- supradiaphragmatic pneumonia. Despite the fever and other signs of the toxic state it is not uncommonly possible to see patients with a clean moist tongue in whom the appetite is maintained or only slight decreased. This distinguishes influenza patients from other patients, for example those with typhoid fever. Usually the tongue is slightly coated, and much less often it is considerably coated with a greyish membrane. There is not always a complete loss of appetite. We did not even once see the "typical" influenzal bluish-white "porcelein" tongue mentioned by certain authors. The patients' stool was frequently normal, and sometimes at the beginning of the disease he was somewhat constipated; from time to time, there was a certain tendency toward diarrhea (pasty stool one or two times on the first or second day of the disease). The abdomen was soft, nontender, and not distended.

In January 1947, during an outbreak of influenza B dynamic investigations were made in our hospital by Shimshelevich using a duodenal tube for obtaining the gastric secretion in a number of young patients in whom no gastrointestinal pathology had been observed in the past. In some patients a depression was found of the secretory activity of the stomach; in others, a hyperfunction of it. These changes depend on the general toxic affect of the infection on the vegetative nervous system, which regulates the gastric secretion, with the predominance of a sympathetic effect in some patients and a parasympathetic effect in others. After the body has been freed of the influenzal toxic state and of its consequences the secretory function of the stomach gradually becomes normal. On repeated examination of these patients two or three weeks later the gastric juice was found to be normal. The acidity decreased in those patients in whom it had been increased at the climax of the disease and increased in those in whom it had been reduced during the acute stage of the disease.

We did not even once encounter any serious complications in the abdominal organs (appendicitis, pancreatitis, etc.) nor any abdominal catastrophes (intestinal obstruction, peritonitis, etc.) which might have required immediate surgical intervention, such as has been indicated in the literature. We believe that these cases were erroneously considered influenza complications by the authors and that the patients probably did not have influenza. It is

possible that the influenza coincided with the surgical disease. In the literature it is possible to find mention of influenza as a cause of many, but not quite all, of the pathological conditions of the organism up to sterility in man (Wolbarst, 1933).

The salivary glands functioned normally. No particular dryness was noted in the mouth. In our clinical material we did not find any inflammation of the salivary glands. However, in our outpatient practice one patient had a unilateral inflammation of the parotid gland which quickly resolved without suppurating.

We did not have any opportunity of observing an increase in the size or other changes in the liver associated with influenza, although, according to the data of Zakstel'skaya (1953), there are considerable changes of it in experiments on animals. Sometimes we noted an aggravation of chronic cholecystitis, and Fridman and Gutman (1952) indicate a moderate enlargement of the liver in certain patients.

Contradictory opinions exist in the literature concerning the condition of the spleen in influenza. Certain pathologists (Meier and Bernhardt, 1918) and clinicians, for example, Koltypin (1923), Sen (1923), Shotter (1927), Andronov (1928), observed an enlargement of the spleen in influenza; others, for example, Tushinskiy (1943), Brauder (1949), Bilibin (1950) did not find this.

N. F. Filatov (1883) pointed out that influenza in children occurs in the majority of cases without an enlarged spleen. The author concluded that the enlargement in the spleen is usually evidence against the existence of influenza in the patient. In the textbooks on children's diseases which were published later he repeats that the liver and spleen are not noticeably enlarged in influenza.

In our observations we have not been able to confirm the statements of certain authors as to the enlargement of the spleen in many cases of influenza, because this has been noted rarely. In the last outbreak of A-double-prime influenza an enlargement of the spleen was noted in three to six percent of the patients.

Kidneys and Urinary Tract. In influenza changes are frequently observed in the condition of the kidneys, as in any other infectious disease, accompanied by a more or less pronounced fever. In the literature there is a large number of works devoted to the problem of the condition of the kidneys in this disease. Some authors, for example, Topchan (1929), Tseytel' (1935), Rozenberg (1931, 1934) and a number of others mention that they have observed acute nephritides and other serious changes in the kidneys and urinary tract produced by influenza (Cook, 1926-1927; Hantschmann,

1933). Other authors believe that the kidneys in influenza are not essentially involved. Nephritis in influenza is a rare phenomenon, and thereby only a transitory albuminuria can be observed (Knack, 1918). Slinchikov (1922), for example, did not observe any cases of nephritis which might be related to influenza. The same contradictory nature of opinions exists also in the evaluation of the frequency of kidney disease in influenza (from 0.029 to 45 percent). This is apparently related to what specifically is considered a kidney complication: more or less serious changes or a brief, febrile trace albuminuria.

Frankfurt and Merzon (1952) observed an increase in glomerular filtration and tubular resorption (by the creatinine test) in influenza. Because the diagnosis was based on the clinical picture and hemagglutination reaction there can be no complete assurance as to its correctness.

Complications have also been described in the urinary passages -- pyelitis, cystitis (Topchan, Delicati, 1927; Podvinek and Pollak, 1929; Weijtlandt, 1929).

There are also disagreements on the matter of the diazo-reaction in influenza.

From the descriptions it is not always clear whether influenza or an acute upper respiratory disease preceded the renal changes. For example, a case history taken by Topchan (1929) causes us to doubt the influenzal origin of the disease. The patient, who suffered from cystitis was cured in eight days. Several days after recovery, she developed, according to the author's conclusion, general signs of influenza (no mention is made in the article as to what these signs were). The patient was sent to the urologist with complaints of frequent painful urination. The temperature was 37.4°-37.7°. The diagnosis of cystopyelitis following influenza was made, which latter disease had apparently caused an exacerbation of a colon bacillus infection. We believe that an exacerbation of the cystitis occurred in the patient followed by an ascending cystopyelitis. There was certainly no influenza here. The internist would have done the correct thing if he had refrained from making a diagnosis of influenza and had put the patient under the observation of the urologist.

The differences of opinion in the evaluation of kidney and urinary tratt signs and of the urine in influenza apparently are related to the nature and characteristics of the cause of various epidemics, the type of patients who come under the observation of various observers and the severity of the disease.

At the climax of influenza not uncommonly albuminuria in traces, occasional casts, and microhematuria are observed (the latter, for example, was noted during the out-

break in March-April 1949 in approximately 64 percent of the patients at one plant medical institute, which was possibly associated with the urotropine used for treatment). Less often, the presence of a large quantity of protein and a large number of erythrocytes in the urine may be noted in influenza. The urinary changes usually disappear with the decrease in temperature and the improvement in the condition of the patient.

We do not consider a slight, transitory albuminuria a renal complication.

Sometimes pyelitis is observed in influenza. More often, the course of chronic kidney diseases, kidney pelvis diseases and diseases of the urinary bladder are aggravated. We have not had any opportunity of observing acute nephritis as a complication of influenza. Statements in the literature indicating that influenza is the cause of nephritis apparently represent diagnostic errors wherein acute catarrhs of the respiratory tract were taken for influenza. The urine (reaction, specific gravity, etc.) showed no abnormalities.

Sexual Organs. In the literature there are many statements of changes and complications in the sexual organs in influenza, frequently very serious (Bugbee, 1919; Serdyukov, 1948). Thus, premature menstruation, abortions, and premature deliveries are described. The latter constitute a great danger and were accompanied by a considerable mortality rate during the "Spanish Flu" pandemic of 1918/1919. (L'vov, 1890, Schmitz, 1919; Litwak, 1932). These were caused by hemorrhagic endometritis with discharge of the ovum, and hemorrhages into the uterine cavity (Litwak). Vulvovaginitis (Mityukevich, 1931), bartholinitis, and purulent inflammation of the ovaries have been described in women, in men, orchitis, epididymitis, prostatitis (Topchan and others), seminal vesiculitis and inflammation of the vas deferens leading to sterility (Engelen, 1922; Mendel, 1923; Frehse, 1932; Wolbarst, 1933; Fossel, 1938). As proof that the complications were the result of influenza the authors refer to anamnestic data, sometimes four-six years old, the isolation of the Bacillus influenzae (Mityukevich, Topchan, and others), and the tremendous martality rate of pregnant women during 1918-1919 at the time of the "Spanish Flu" epidemic.

The cases presented by the authors are not always convincing in the sense of correctness in considering them of influenzal origin. We should like to point out, for example, the observations of Baker (1927), who describes influenzal epididymitis. This epididymitis occurred after so-called "influenzal diseases". The basis for this diagnosis was a fever which might also have been produced by a

purulent process in the deeply situated organs, for example, in the seminal vesicles. Of the 50 cases of vulvovaginitis described by Mityukevich in children as complications of influenza and in which the Bacillus influenzae was isolated, influenza was found in the history of only 32 children.

In our extensive material on influenzal diseases (men were predominant among the patients) we did not observe any changes in the male sexual organs associated with these diseases. We doubt the influenzal nature of the complications given by various authors pertaining to the sex organs in men. As far as the complications in female sex organs are concerned we have sometimes observed premature menstruations. There were no abortions. However, our observations are not significant in this connection, because our material was too small.

Lymphatic Apparatus. In influenza changes are sometimes observed in the lymphatic apparatus (Lange, 1927). Pathologists have found them on section. Thus, according to Pozharisskiy (1921), hyperplasia of the lymphatic apparatus, acute enlargement of the lymph nodes, and the presence of status lymphaticus in a number of cases was characteristic. The lymph nodes of the neck and of the lung roots are particularly affected. They are numerous, enlarged, and soft; on section they are of a greyish-red or red color. Abrikosov (1950) also notes an acute hyperplasia of the lymphatic apparatus in influenza, chiefly of the paratracheal and cervical lymph nodes. Davydovskiy, Dvizhkov and Chalisov (1938) indicate that the lymph nodes of the neck and mediastinum are enlarged, succulent, of red color, which indicates a hemorrhagic lymphadenitis, sometimes with suppuration and a transition to a mediastinal abscess.

There are various communications in existence concerning the lymphatic apparatus. In some it is pointed out that the lymph nodes are for the most part not enlarged (Kraus, 1911); in others (Brash, 1918; Schmiden, 1919; Sen, 1923; Plate, 1929; Franke, 1930), mention is made of swelling and tenderness of regional lymph nodes with hyperemia of the pharynx, and sometimes soft, enlarged submaxillary lymph nodes.

We have frequently encountered patients (up to two-fifth of the cases) with somewhat enlarged submaxillary or cervical lymph nodes. The nodes were sharply defined, firm and not tender. We did not observe any fresh lymphadenitis. Evidently such cases were residuals after pathological processes in the mouth and throat associated with a regional lymphadenitis. At autopsy of four of our patients no changes were noted in the lymph nodes.

Locomotor Organs. Pathological phenomena in the organs of locomotion (bones, muscles, joints) represent an expression of the toxic state in the acute stage of influenza. The patients complain of a feeling od depression, rheumatic type aches, pains in various parts of the body, in the extremities, lumbar region, in the back, in various muscle groups, in the calves, in the chest, cervical or spinal muscles, etc., and less often in the joints.

In the literature there are many statements as to the involvement of organs of locomotion, but there are usually no objective changes noted. Sometimes more severe diseases are observed accompanied by objective changes. A number of authors note that in influenza pains in the muscles are encountered frequently. At autopsy, hemorrhages are sometimes found in the muscles, and less often a waxy degeneration or abscesses. Pain in the joints is frequently encountered; less often, they swell up, and even less often a suppuration is observed in the joints. Diseases of the bones, periostitis, and osteomyelitis occur rarely. Dobreytser (1937) mentions arthritis observed during influenza outbreaks in 1936; Arsen'yev (1938) and Nikulina (1938) described arthritis and osteomyelitis in influenza. Arsen'yev observed perichondritis and bursitis.

According to descriptions of the clinical picture of arthritis existing in the literature, a number of joints are involved as a rule, large and small, simultaneously, and sometimes they are involved in sequence. The tendon attachments of the muscles are found to be involved. These complications disappear after several days; in rarer cases the joint changes last up to three weeks. An allergic origin is ascribed to these cases of arthritis (Ionin, 1940), although it is not known what specifically constitutes the allergen in this case (Hildebrandt, 1919; Redwitz, 1921; Weil, 1929; Munro, 1930; Gundel, 1934). Rozenberg and others write about secondary infections in the form of osteomyelitis and purulent arthritis in influenza.

By far not all of the authors are convinced of the influenzal origin of the involvement of the locomotor apparatus. The diagnosis of influenzal arthritis is usually made tentatively and is based on the occurrence of arthritis shortly after the case of influenza.

Franke (1930) has written a great deal about the changes in the locomotor organs (inflammation of the gastrocnemius muscles, involvement of bones and joints). He observed such changes also in acute influenza, but erroneously considered them expressions of chronic influenza (periostitis of the shaft of the tibia, lesion of the inner condyle, of the lower epiphysis of the femur, etc.).

From time to time in our hospital arthralgias and

sometimes myalgias have been noted in the acute stage of the disease, in addition to the general toxic signs in the form of depression and rheumatic pains, in various bones, muscles and joints; these were observed in 60-81 percent of the cases of influenza. On the average, the complications in the locomotor organs (for example, lingering pains in the muscles and joints at the time of the decrease in temperature), constituted about three percent.

65. The Nervous System and the Sense Organs

The Nervous System. The influenza virus affects not only the respiratory tract but also the nervous system, chiefly the vegetative nervous system. Toxic signs observed in influenza -- chill, fever, depression, etc. -- attest to the involvement of the central nervous system. The patients are frequently apathetic, drowsey, "as in encephalitis". According to the data of Stuart-Harris and coauthors (1938), insomnia was noted in 32 percent of them. Sometimes ephemeral cerebral and meningeal signs are observed. Certain clinical signs, for example, facial hyperemia, hyperemia of the mucous membranes of the respiratory tract, eyes, tendency toward perspiration, increased secretion of the nasal mucosa, changes in the capillaries, hypotension, etc. represent the result of vegetative nervous system involvement, which is expressed in an increase in its parasympathetic and a depression (Medvedev, 1954) in its sympathetic functions. At the climax of the disease increased reactions are observed from various peripheral nerves. This was established in the Soviet Union clinically by Ivanova (1950) during an influenza B outbreak 1946-1947; by Seregina and Zimovskiy during an influenza A-prime outbreak in 1949; and through the works of Davidenkov and his co-workers (1951, 1953) in the material of 1949, 1950 and 1952. The authors frequently noted neuralgias of various branches of the trigeminal nerve, intercostal and other nerves, involvement of the facial nerve as well as radiculitides, different kinds of hyperesthesias and anesthesias, Horner's syndrome, anisocoria, and a whole series of other nerve signs the majority of which disappeared simultaneously with the decrease in temperature and with the cessation of general toxic signs. Man'kovskiy, Mints and Raygorodskaya (1952) write about the exacerbations of chronic nervous diseases in influenza and of relapses and provocations of nervous system infectious processes of other etiologies (multiple sclerosis and others).

In a number of cases the nervous system signs are expressed to such a marked degree that certain authors speak of a special nervous form of influenza. We consider

this incorrect, because any form of influenza occurs with more or less pronounced nervous components and the general toxic state characteristic of any form of influenza is expressed in nervous manifestations observed in it. The toxic signs constitute the principal general signs of influenza, in comparison with which the local catarrhal lesions at the beginning of the disease become less prominent.

A tremendous literature exists concerning the state of the nervous system in influenza (see Postovskiy, 1904; Geymanovich, 1919; Burckhardt, 1926; Frank, 1927; Baliyev, 1927; Ziman, 1928; Belousova, 1928; Kauders, 1932; Dufourd, Hurez, 1932; Urechiac, 1933; Pette, 1934; Dekhterev, 1934-1938; Dragonesco, Fason, 1934; Korganov, 1934; Tarasevich and Mikheyev, 1934; Karchikyan, 1936; Vasil'yeva, 1936; Zelkan and Kurzon, 1936; Shevelev, 1936; Leshchenko and Vasilenko, 1937; Shenderovich and Droznina, 1937; Khazoanov, 1937; Gamarnik, 1937; But, 1938; Glinchikov, 1938; Vyyasnovskiy, 1940; Rossiyskiy, 1942; Popova, 1945; Vizen, 1947; Rubnich, 1948. Of the latest authors mention may be made of: Kul'kova, 1951; Bogolepov, 1951; Davidenkov and coworkers, 1951-1953; Litvak, 1952; Bochkarev and Butman, 1953; Wegmann, 1953; Pratusevich, 1953; Sylla, 1953). Many facts are presented in it which constitute evidence of the possibility of all kinds of psychic manifestations in references made here to headache, not uncommonly dizziness and a syncopal state, sometimes exitation and insomnia. Glinchikov observed patients with clouding of consciousness, furibund delirium and hallucinations. Sometimes, on the other hand, a severe somnolence is noted which can change over into an actual coma, great weakness and general depression, aching pains in the calves, thighs, sacrum, and joints. Neuralgic pains are observed in the intercostal nerves, in the sciatic and trigeminal nerves, hyperesthesia, sometimes paresthesia or anesthesia of the skin, disturbances in the gustatory and olfactory sensations. Not uncommonly, severe inflammatory and degenerative lesions develop in the form of neuritis, polyneuritis or cerebral of spinal cord diseases (purulent or serous meningitides and encephalitides). The nervous system lesions produce the most variegated clinical picture depending on the localization and intensity of the process -- lemiplegia, monoplegia, aphasia, paralysis of the facial and hypoglosal nerves. Less often, involvement of the oculomotor nerves is observed. These paralyses may pass over very quickly, which is characteristic of influenzal encephalitis. The spinal cord involvements are of the same nature as the cerebral.

This, in general, is the almost exhaustive picture of the phenomena which occur in influenza and which may be observed in the central and peripheral nervous systems.

There is no need for any special discussion of the data of various authors concerning the nervous and pyschiatric complications in a number of patients caused by the influenzal infection. Caution should be observed in stating that encephalitis and myelitis are of an influenzal nature, since virologically this has not been proved, and epidemiological or anamnestic data have constituted the basis for this conclusion.

Certain authors, including the clinical neuropathologists (for example, Aronovich, 1934), doubt the existence of influenzal encephalitides and other nervous system diseases which have not as yet been proved by the laboratory. In 1944 and on the same basis we criticized authors who pointed out the connection of the nervous and many other diseases with influenza. The diagnoses of the authors was frequently based on inexact anamnestic data of a history of influenza. In this connection, the work of Zhdanov and Rutshteyn (1950) "on the so-called postinfluenzal infections of the nervous system" (encephalitis, serous meningitis, arachnoiditis, encephalomyelitis, meningomyelitis, menigoradiculitis, etc.) is interesting. Based on laboratory virological investigations the authors deny the influenzal nature of many nervous diseases considered to be complications of influenza.

Nevertheless, complications of the nervous system which develop during the course of influenza can sometimes be observed. Among them are very transitory neuralgias, which are eliminated with recovery, and more prolonged inflammatory processes. We have not observed any serious diseases of the nervous system like encephalitides or meningitides, etc. In the literature there are scattered indications of virological confirmations of these diagnoses (Partusevich, 1953; Vashchenko and co-authors, 1955). In previous works the diagnoses were based exclusively on the clinical data, because the etiological role of influenza had not been proved.

In the great majority of cases there was red dermographia in influenza, and much less frequently it was white.

We have had an opportunity to observe herpes zoster in influenza.

Sense Organs. The sense organs suffer considerably in influenza. Definite signs of involvement of them are observed, and sometimes also serious complications.

Changes in the eye observed at the climax of the disease (cutting feeling, pains in the eyes, etc.) occur simultaneously with the disappearance of the toxic state. We have not observed any eye complications (with the exception of an extremely rare dacryocystitis) even once.

Certain authors describe various complications in the

eyes in influenza (Ewerbusch), 1890; Löwenstein, 1918; Braunshteyn, 1919; Berberov, 1929; Sotnikov, 1929; Levitskiy, 1930; Jaeger, 1931; Khordas, 1935; Prokhorova, 1938; Rozenberg, Rossiyskiy, 1939). However, the specific etiology remains unproved. Faktorovich (1928) expressed doubt as to the association of these complications with influenza. Bogdanovich and Merkulova, in the article "Influenzal Complications in the Eye," deny these complications on the basis of virological research.

In influenza, as a rule, a stimulation of the tunica conjunctiva bulbi is noted as well as conjunctivitis of different degrees. In addition, there are pains on movement and pressure on the eyeballs, lacrimation, photophobia and scleritis.

In outbreaks of influenza A, A-prime and B we have not observed any serious complications in the eyes. Pains in the eyes, conjunctivitides and other changes in the eye were noted in 53-80 percent of the patients with influenza during the outbreak. We do not know of any eye complications after discharge of the patients from the hospital.

Organ of Hearing. The ears, and specifically the middle ear, have a direct connection with the upper respiratory tract. The inflammatory process can extend per continuitatum from the upper respiratory tract to the eustachian tube and to the mucous membrane of the middle ear. Any infection can penetrate from the nasal pharynx into the middle ear and produce various changes there. There is nothing surprising in the fact that the organ of hearing, usually the middle ear, becomes the arena of various inflammatory processes complicating influenza.

The ear changes occupy an important place among all the complications observed in influenza.

In the literature there are many works which discuss this problem in detail. The authors have listed different kinds of complications of the auditory apparatus which, in their opinion, are encountered in influenza and are connected with it (Beyer, 1948; Denker and Albrecht, 1936; Kuperman, 1936; Davydov, 1938; Preobrazhenskiy, 1945, and others). These are purulent and hemorrhagic otitis, hemorrhagic complications -- abscesses, thromboses of the blood vessels and others (Konchalovskiy, 1927; Rozenberg, 1934), acute catarrh of the mucous membrane of the eustachian tube and middle ear, and labyrinthitis (Kompaneyets, 1929). According to the data of Nadzharov (1933), in the great majority of patients in whom there were complications in the middle ear nasal respiration was difficult. At various seasons the complications in the middle ear in influenza were noted in 16-35 percent of the cases.

We should like to present the work of Wodak (1929) as

an example of unfounded diagnosis. This author describes eight cases of disease of the inner ear (vestibular apparatus of the eighth nerve) with Meniere's syndrome following influenza (questionable). In certain cases there was mention of influenza or catarrhal signs in the history; in other cases there was none. Nevertheless, in view of the great number of these diseases the author believes that these complications are of an infectious origin, and as long as it is unknown which infection causes them influenza comes on to the scene. In conclusion, contradicting himself, the author considers it possible to speak of influenza of the eighth nerve, although he had placed a question mark in the title of his work concerning the influenzal origin of the cases which he described.

It may be supposed that in many cases of disease of the auditory apparatus of "influenzal etiology" the real cause of the disease was not found by the physician.

According to our data, not uncommonly complications are noted in influenza in the auditory organ in the form of eustachitis, catarrhal otitis, laryngitis, sometimes hemorrhagic purulent inflammation of the middle ear. Thin vesicles filled with hemorrhagic contents are observed in the auditory canal. The ears suffer particularly frequently in younger children. This is explained by the fact that the eustachian tube is shorter and broader in children than in adults. At the climax of the disease complications are also found in the vestibular apparatus (of toxic origin).

We have not had the opportunity of observing any complications in the acoustic nerve, although there is mention of this in the literature. A certain (temporary) decrease in hearing may be observed as a result of the toxic state or of complications in the ear. In 1939, acute purulent inflammation of the middle ear occurred in less than one percent of the cases. In 1940, complications were observed in the ears in 6.5 percent of the cases; of these there were the following: eustachitides -- in almost four percent; catarrhal otitides, less than two percent; purulent otitides, less than one percent; and external otitides, also less than one percent. In subsequent epidemics complications were also observed in the auditory organs -- eustachitides, catarrhal and purulent otitides (seven to nine percent). We have not encountered any acute involvement of the inner ear. Once we observed a case of a reduction in hearing on the background of otosclerosis.

From the data presented it is seen that various influenza epidemics are distinguished from one another by the nature of the complications which occur in the auditory apparatus. This difference is insignificant, and we have not had the opportunity of observing any serious complications

in the auditory apparatus requiring operative intervention, for example mastoiditis.

Olfaction. The Nose. In influenza olfactory disorders may be observed: losses of olfaction -- anosmia, and a weakening of olfaction -- hyposmia (Leichtenstern, 1896; Collet, 1904; Letnik, 1940; Rozenberg, and others). More than a hundred years ago, Magaziner in 1836 reported on the olfactory and gustatory changes in influenza. According to Kompaneyets (1942), in infections, particularly in influenza, essential hyposmia or anosmia may be observed which occurs under the influence of a destruction of olfactory cells and nerve fibers as well as of the olfactory area in the nose.

Acute antral sinusitis and frontal sinusitis often encountered in influenza may be accompanied by anosmia. In influenza a transitory anosmia can sometimes be observed as a result of edema of the mucosa of the middle concha and the presence of an increased quantity of discharge in the nasal cavity covering the olfactory foramina. The olfactory disturbance may be the result of a toxic process and perhaps of a pathological one in the olfactory nerve, as has been shown in the laboratory of Professor Vinnikov (Tret'yakova, 1952) in an influenzal infection of white mice.

We have not observed any prolonged or considerable olfactory disorders in our patients and have seen rather only a temporary and very transitory decrease in this function.

Taste. Indications of a gustatory disturbance in influenza are encountered rarely in the literature. Mention of a change in taste in influenza has been made by Cramer, 1930, Franke and Magaziner. Rozenberg also notes a gustatory disturbance in influenza; Barkhash (1939) indicates a loss of taste. Hellmann (1929), and Timofeyev (1941) have observed a gustatory disorder in diseases of the respiratory organs, middle ear, etc.

Not uncommonly, influenza patients complain of a reduction in taste sensation, of an absence of it, of tastelessness of the food at the climax of the disease. These signs are usually associated with a loss of appetite and possibly with a toxic involvement of the gustatory nerves. They disappear rapidly with the improvement of the general condition and with the mitigation of the toxic state.

We have not had any opportunity to observe any serious taste disorder in influenza, and only in rare cases have we noted any temporary reduction of it at the climax of the disease.

Touch. We have not seen any cases of involvement of

the tactile sensations in influenza. True, we have not made any special study of this problem, and it is possible that touch sensation also suffers somewhat at the climax of the disease.

66. Blood

The blood morphology in influenza does not constitute such a solid support in connection with the diagnosis as in other infectious diseases, for example, in scarlet fever, typhoid fever, malaria and croupous pneumonia, etc.

A description of the blood picture in influenza is given in the textbooks and monographs (Glinchikov, 1922, 1938; Konchalovskiy, 1927; Nipperdey, 1929; Freyfel'd, 1934, etc.). There are many special works, both old and new, devoted to this problem (Kolman, 1890; Rosenow, 1918; Kronberger, 1919; Hildebrandt, 1919; Kryukov, 1920; Hess, 1925; Sokolova, 1926; Braude and Freyfel'd, 1930; Kresse, 1935; Shchedrovitskiy and Levitina, 1937; Vorobeychik, 1937; Yegorov, 1937; Wetzel, 1937; Pean, 1938; Poppel' and co-authors, 1948; Gertsenberg, 1940; Nechayev and Chalkina, 1941, and many others).

There is considerable literature on the white blood element in influenza. However, the authors have not reached any unanimous conclusions. Some have found a leukocytosis; others, a normal white blood count or even a leukopenia (Jagic, 1918); still others, a variegated picture; still others, leucocytosis followed by leukopenia; and still others, leukopenia followed by a subsequent normal white blood count or leukocytosis (Lewy, 1918). The same contradictory situation exists also in descriptions of various components of the blood (Arneth, 1920; Hirschfeld, 1930).

Aside from methodological errors (Reiche, 1920; Ufsepp, 1927), this contradictory situation is explained by the absence of any possibility of making an accurate etiological diagnosis of influenza at that time.

The negative viewpoint of the diagnostic value of the white blood picture in influenza taken by current foreign authors, who have used virological investigations for confirming the diagnosis of influenza, is incomprehensible. For example, Stuart-Harris and co-authors, who in 1938 presented a table of the differential diagnostic characteristics of influenza and "febrile" (we call it "acute") catarrh of the respiratory tract, wrote that the blood cannot serve as a feature for distinguishing influenza from acute catarrhs. Nevertheless, after a careful examination of the material which they presented a difference may be seen between the white blood picture in influenza and that in acute catarrhs of the respiratory tract.

It is to the credit of Soviet authors that they have developed the problem of the diagnostic significance of the white blood in influenza. First of all, mention should be made of Korovin (1937, 1941) in the hospital directed by Tushinskiy, and then Kolyaditskaya (1948) and Dubnyakova (1952, 1953, 1954) from the influenza hospital of the Institute of Virology of the Academy of Medical Sciences USSR. In their investigations they have described the blood picture in detail found in influenza and acute catarrhs of the respiratory tract, with a dynamic examination of it and constant virological control.

Only recently have foreign investigators directed attention to the study of the blood picture in influenza. Here, for example, we have the observations of Siede and Schneider (1953), who after many years have confirmed the work of Soviet investigators concerning the state of the white blood element in influenza. It is curious that these authors indicate a relationship of foreign physicians to the investigation of blood in influenza. They write that Focht, during an outbreak of influenza in 1951 and after the interrogation of 52 physicians, concluded that "there is no typical blood picture in influenza". In the opinion of these authors, the cause of the disagreement in the evaluation of the white blood picture in influenza is the fact that the blood of influenzal patients was examined during various stages of the disease and that the bacterial complications of influenza had not been taken into account sufficiently.

This comment by Siede and Schneider is absolutely correct, which was pointed out by Korovin in 1937 after he had studied the white blood picture in the 1936 influenza outbreak.

We also managed to note in 1941 that the white blood picture in catarrhs of the respiratory tract is different from that in influenza.

On the basis of the investigations of Korovin, Kolyaditskaya, and Dubnyakova it has been established that influenza occurs with leukopenia in approximately 25 percent of the cases as early as the first day of the disease. While at the beginning of the disease the leucocyte count is normal or there is even a slight leucocytosis, by the third day of the disease the leukopenia is noted in approximately 75 percent of the patients. In the other cases the absence of leukopenia is usually associated with complications or with associated diseases which occur with the leucocytosis and in which the blood picture is altered. Note should be made particularly of the influenza A-prime outbreak in 1952 wherein a marked leukopenia was observed in the majority of patients as early as the first two days of

the disease (Dubnyakova, 1954).

During the period of the fever an absence or a decrease in the eosinophil count, neutropenia (for the most part in parallel with the leukopenia), a relative lymphocytopenia, usually beginning with the first to second day of the disease, and a relative lymphocytosis beginning with the third day or later (an absolute lymphocytopenia may be observed), monocytosis, a certain neutrophilic shift to the left, toxic granulation of the neutrophils, early thrombocytopenia are noted; sedimentation rate usually remains within normal limits.

Our observations during the influenza outbreak in 1940 and in 1943-1944 are in agreement with the data of Korovin, Kolyaditskaya and Dubnyakova.

Complcations and associated diseases may alter the blood picture depending on their nature and their influence on leukopoiesis, on various formed elements, and on the sedimentation rate.

With the improvement in the patient's condition eosinophils appear in the blood, the shift to the left becomes less pronounced, the toxic granulation of the neutrophils decreases, and there is an increase in the number of lymphocytes. During the period of convalescence the blood becomes normal, which may drag on for several weeks.

Concerning the matter of the mechanism of leukopenia observed in influenza we have the work of Schnetz and Greiff (1937, 1938) who simultaneously investigated the peripheral blood and a bone marrow smear taken by sternal puncture from 14 influenzal patients. Thereby, they found leukopenia in the uncomplicated cases both in the bone marrow smear and in the peripheral blood. These observations confirm the viewpoint that leukopenia in influenza is the result of an inhibition of leukopoiesis through a toxic influence of the influenza virus. When the organism is free of the toxic influence of the influenza virus the leukopenia disappears. In complicated cases produced by pneumococci, streptococci and other microbes which stimulate leukopoiesis, different results may be obtained depending on when the complication occurred: whether at the beginning of the disease, at which time the toxic effect of the influenza virus is in force, or later, at which time the effect of the virus has stopped. In the latter case, the usual reaction in the white blood is expressed in leucocytosis. In the former case two opposite factors interact: on the one hand, inhibition; and on the other, stimulation of leukopoiesis. In sum, in some cases a normal leucocyte count may be obtained; in others, a moderate leukopenia or moderate leucocytosis.

We find mention of the condition of the red blood in the works of a number of authors. Some of them note that

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the red blood element shows no changes nor abnormalities (Troitskiy, Kuznetsova and Podlutskaya, 1919; Vorobeychik, 1937, and others); others find a decrease in the erythrocyte count and in the quantity of hemoglobin (Shatilov, 1919; Kireyev, 1927, and others). Certain authors have sometimes found a considerable increase in the erythrocyte count. Thus, Naegeli (1923) points out that the erythrocyte count reached 7,500,000 in connection with perspiration, with the loss of fluid by the body, with hemoconcentration. Arndt (1933) also observed erythrocytosis (up to 6,800,000). He believes that the erythrocytosis is associated with the influenza virus, the toxins of which act simultaneously -- inhibiting leukopoiesis and stimulating erythropoiesis. At the same time, the quantity of hemoglobin does not increase, and the color index drops.

Stuart-Harris and co-authors observed an erythrocyte count of from 4,000,000 to 6,000,000 and a quantity of hemoglobin of from 82 to 102 percent in influenza patients.

We have not observed any particular changes in the red blood element and have seen only a slight increase in the erythrocyte count and in the quantity of hemoglobin.

Arndt found a delayed sedimentation rate in cases of influenza during the toxic state occurring with leukopenia and erythrocytosis (constantly in the latter), and an accelerated sedimentation rate in severe inflammatory processes.

Gertsenberg, Poppel', Ol'yak and Pokras (1940) noted an accelerated sedimentation rate.

Balkovskiy, Moskvin and Osetrov (1937) obtained a normal sedimentation rate in 50 uncomplicated cases of influenza; in complicated cases, an accelerated sedimentation rate.

With respect to the sedimentation rate values in our clinical material the following data were obtained during various outbreaks; under 10 millimeters in an hour, in 40-87 percent of the cases; between 10 and 15 millimeters, in 6 to 27 percent; and over 15 millimeters, in 7 to 35 percent of the cases (an increase to 30 millimeters in an hour was noted only in isolated cases); with the fall in temperature the sedimentation rate returned to normal.

Zakrzhevskiy, Perla and Sasiko (1952) present approximately the same blood picture in influenza as was given by Korovin, Kolyaditskaya and Dubnyakova. During the convalescent period the blood does not become completely normal. In the majority of patients the sedimentation rate is accelerated, and during the recovery period it does not return to normal. The same blood picture was noted by the authors both in cases of influenza with a positive hemagglutination reaction with throat washings and in cases with

a negative hemagglutination reaction.

The following data have been obtained from bone marrow puncture at the climax of the disease: an increase in the percentage of neutrophilic myelocytes, a decrease in the percentage of normoblasts, an increase in the leukoblast index, a reduction in the maturation indices of the granulocytes and erythroblasts, and the occurrence of hypersegmented megakaryocytes. During the recovery period a tendency toward normalization of the bone marrow is observed.

The data concerning the condition of the platelets in influenza are very sparse in the literature. Certain authors (Chistovich, 1906) note that the platelet count decreased during the climax of the disease, and with recovery it is very much higher than normal. Sometimes, in the same individual a great difference in the thrombocyte count is observed. According to Alder (1920), the thrombocytes in influenza remain normal. According to the data of Dubnyakova (1954), the platelet count decreases from the very beginning of the disease.

In the opinion of Troitskiy and co-authors, the blood viscosity is normal; according to Alder it is first reduced and then increased.

Leichtenstern mentions solitary cases in which hemoglobinuria developed after influenza which were observed by Baümler as well as cases of pernicious anemia which were observed by Reider (1892), and of leukemia.

67. The Endocrine System and Metabolism

Endocrine System. Konchalovskiy, in his monograph on influenza (1927), mentions the fact that the adynamia, hypotension, lumbar pains, white dermographia (we have observed white dermographia rarely in influenza; more often, we have seen red dermographia -- F. E.) observed in influenza are related to the involvement of the suprarenal glands. Rozenberg (1934) writes about the changes in influenza which occur in the thyroid, thymus, pancreas and suprarenals. Rossiyskiy, on the basis of data in the literature and his own observations, devotes a number of articles (1939, 1942) as well as a chapter in his monograph (1942, 1945, 1951) to lesions in the organs of internal secretion in influenza. Suprarenal involvement has been described by Kovacs (1929).

Autopsy data in people who have died from influenza show evidence of changes in the organs of internal secretion. Abrikosov (1941) writes that hyperemia and sometimes hemorrhages into the suprarenal glands, a decrease in the concentration of lipoids in the cortex and a weakening of the chromaffin reaction of the medullary substance are noted in influenza. In children under the age of two Karlinskaya

(1935) notes a thickening of the connective-tissue stroma, a degeneration of Hassall's corpuscles and a decrease in the size of the follicles in the thymus gland. In the suprarenal cortex she found foci of necrosis, a reduction in the quantity of lipoids; in the medullary layer, hemorrhages; and in the pancreas, a proliferation of the connective-tissue stroma and atrophy of the lobules. The degree of changes in the organs mentioned were more pronounced in the thyroid gland; less, in the suprarenal glands, and even less in the pancreas. Hammar (1923) investigated 65 cadavers. The ages of the patients who had died were from 17 to 35 years. Death had occurred during the acute period of influenza on the 5th to 16th day of the disease, which had in the majority of cases been complicated by pneumonia. The author notes a reduction in the weight of the thymus gland parenchyma in 40 cases; in 25 cases the weight was at the lower limit of normal. The number of Hassall's corpuscles in 36 cases was reduced. The decrease in the weight of the parenchyma did not depend on the general nutrition of the body, because it was noted in quite well-nourished persons. The author has not succeeded in convincing us that the condition of the thyroid gland which he found was associated with the influenzal infection rather than with the complications from which his patients died. He indicated the development of diabetes mellitus after influenza. In his opinion, such cases are not particularly rare. Lereboullet and Gourney (1927) adhere to a different point of view. However, in patients with diabetes a deterioration of the process may occur in connection with having had an infection and a disturbance in the sugar regulation in the blood. Influenza does not have any more severe course in them. Rowntree (1925) and others associate the development of Addison's disease with influenza. Mosenkis (1952) points out that in influenza there is an abnormality in the blood adrenalin concentration in the great majority of patients in the direction of a decrease or increase in it. On recovery, the concentration of adrenalin returns to normal. The secretory function of the suprarenals is not impaired in influenza, and no parallelism is noted between the blood pressure and the blood adrenalin level.

According to Barkhash (1939), the blood amylase decreases in influenza. There are also indications of the rarity of endocrine involvement in influenza. Thyrotoxicoses are encountered only exceptionally, although the condition of patients with thyrotoxicoses may deteriorate under the influence of influenza (Bourgeois, 1932). We doubt that acute pancreatitis described by Wal and Wetzel (1931) was caused by influenza. The diagnosis of influenza here had little basis: the authors made it on the basis of the

increasing number of cases of influenza in the city and the existence of bronchitis in their patient.

We have not observed any disorders in influenza in the endocrine system, except for a more or less considerable adynamia and hypotension. Incidentally, we do not exclude the possibilities of such a complication in view of the frequently observed cases of hyperthyroidism, which have been noted recently in the hospital after influenza outbreaks. Melikhov (1941), for example, mentions that the cause of the acute form of Basedow's disease is influenza and angina in 38 percent of the cases. Although this percentage was computed on the basis of data taken from case histories, which is very inexact, it is still impossible to exclude completely the effect of influenza in these cases.

Certain authors, for example Atabek (1951), associate the development of diabetes insipidus with influenza. We have observed two cases of diabetes insipidus which were found during the time the patients were in the hospital. One of these patients was hospitalized for influenza; the other for acute catarrh of the respiratory tract. However, it was made clear from the history that in one patient the pathological signs were noted before he became sick with influenza. They occurred following skull trauma. In another patient the connection of the diabetes insipidus with acute catarrh of the respiratory tract was not established either.

Metabolism. There is comparatively sparse information on the metabolism in influenza in the literature (Eyband and Baur, 1949). In Rozenberg's textbook of infectious idseases (1934) we read that "...the metabolism in influenza is subject to an essential disorder. The concentration of blood sugar increases; the respiration and the serous discharge of the respiratory tract give off the odor of acetone. The non-protein nitrogen, the urea nitrogen, creatinine, and uric acids are increased. The urine frequently contains albumin, sugar, urobilinogen and traces of acetone. The diazo-reaction is variable. All these data in connection with the increase of ammonia in the urine lead to the conclusion that in influenza there are signs of acidosis". Rozenberg does not mention the form of influenza which he is referring to -- mild or severe, complicated or uncomplicated, or in which complications he made his observations. In various monographs, for example, those of Glinchikov (1922) and Konchalovskiy (1927) there are no data either concerning the metabolism in influenza.

The sugar curve, according to Gurevich (1937), Erez and Gurevich (1940) is normal in the catarrhal form of uncomplicated influenza; in the toxic form a considerable increase in it is noted. The sugar curve does not return to

the original level even during convalescence.

Shchedrovitskiy and Levitina (1937) observed an increase in the quantity of cholesterol in the blood of influenza patients. Jackson (1928) notes a hypocholesterolemia and hypolipoidemia in influenza.

According to Shchedrovitskiy and Levitina, there is an increased quantity of calcium and potassium salts in the blood in influenza. According to the data of Borevskaya, Gubergrits and Lukantsever (1940), the concentration of potassium, calcium and chlorine does not undergo any changes in influenza. The changes in the chlorine and sodium concentration are not interdependent. Zhupanenko, Erez and Gurevich (1940) found that the chlorine level in the blood and urine stays at the lower limit of normal in influenza, and in the bronchial pneumonias undergoes a marked decrease.

Kofanov (1934) in sporadic influenza found that the basal metabolism was normal or decreased; an increased metabolism was evidence of the existence of some additional disease. Oppel' and Chulkov (1936) found that at the climax of influenza there is a decrease in the oxygen uptake, whereas in convalescents it returned to normal. The increased carbon dioxide excretion after sugar ingestion decreased but to a lesser degree than the oxygen uptake.

The concentration of blood bilirubin and of urobilin in the urine is within normal limits, according to Borevskaya, Gubergrits and Lukantsever. According to Rozenberg, the urine contains urobilinogen. Triger (1932) noted a positive diazo-reaction in 50 percent of influenza patients. Friedman and Gutman (1952) observed the appearance of bile acids in the urine in influenza patients, a decrease in the prothrombin and a pathological reaction with sodium benzoate. In some patients certain pathological signs were found; in others, other signs. The pathological signs occurred in influenza and in the acute catarrhs of the respiratory tract, whereby there was no correlation between the severity of the clinical course of the disease and the pathological reaction of the liver.

Malyarevskaya (1952) indicates an increase in bile acids in the blood of influenza patients.

According to Naegeli (1923), who based his opinion on the works of Alder (1920), the quantity of blood proteins drops to five percent beginning with the first day of influenza, but not constantly. Beginning with the second day, the albumin-globulin ratio is changed, whereby the quantity of globulin can come up to 70 percent. This phenomenon can last for 8 to 14 days.

In 1952, certain works were published on metabolism which came from the hospital of M. M. Gubergrits. According to Bronshteyn and Lifshits (1952), the total protein re-

mains normal with a decrease in the quantity of albumin and an increase in the quantity of albumin and an increase in the quantity of globulin. Such an albumin-globulin ratio obtains also during clinical recovery. Fibrinogen, which increases at the beginning of the disease, decreases in the majority of patients during recovery.

The concentration of sodium chloride increases and remains at a high level during recovery. Potassium, calcium and magnesium are reduced at the beginning of the disease and return to normal during recovery.

The authors observed similar changes in influenza and acute catarrhs of the respiratory tract. Doubts arise automatically as to the accuracy of diagnosis of these diseases, particularly since cases were classified under the diagnosis of influenza which had a positive hemagglutination reaction with throat washings, which is not convincing.

Kamenetskiy and co-authors (1952) found a disturbance in the vitamin metabolism in influenza (vitamins A, B_1, B_2, and C). These disturbances which were expressed to the same degree in influenza and in acute catarrhs of the respiratory tract were apparently associated with an increased vitamin requirement by the body tissues and a disorder of the metabolic processes under the influence of infection. A supply of vitamins A, B_1, B_2, and C is required for treating the infection.

According to our data, the concentration of nonprotein nitrogen in the blood of patients with influenza varied: 1) normal, 2) at the upper limit of normal, and 3) somewhat above normal (maximum 43.2 milligrams percent). The concentration of blood sugar on a fasting stomach not only was not increased (maximum 97 milligrams percent) but in certain cases it amounted to 70-72 milligrams percent. The sugar curve frequently did not come up to the original level 2 1/2 hours after a sugar load. Hypocholesterolemia was observed in all patients. The concentration of chlorides in the blood was usually increased. The blood bilirubin, with rare exceptions, did not go beyond the limits of normal. The qualitative reaction was direct. The urine urobilin remained within normal limits. The quantity of urine was somewhat decreased at the climax of the disease in connection with a general febrile condition and partly in connection with increased perspiration.

It is possible that hypocholesterolemia and partly also hypoglycemia in our patients, who were observed during the war years, were not associated with influenza but rather depended on the general nutritional and metabolic conditions.

The similar picture which we observed in cases of

"cold" catarrhs serves as the basis for this assumption.

The McClure-Aldrich test, which we performed at the climax of the disease in uncomplicated cases, did not show any abnormalities. Only twice did we obtain an acceleration of it in cases complicated by pneumonias: once it lasted 20 minutes; the other time, less. In the latter case we were dealing with a primary influenzal hemorrhagic pneumonia which terminated fatally.

68. General Complications

Influenza Relapses. Chronic Influenza. Speaking about the condition of various organs, we have been discussing the changes and complications which may be observed in them in connection with influenza. The most frequent and severe complications are observed in the lungs. Not uncommonly, there are cases of sinusitis as well as otitis (in children particularly), and many other complications. We have already pointed out that influenza involves the weakest places in the body, provoking and exacerbating the chronic processes. Influenza predisposes to other diseases in connection with anergization and a reduction in the body's resistance.

The question of exacerbation of a tuberculous process under the influence of influenza has not met with unanimous agreement. Whereas some authors point to an exacerbation of tuberculosis in connection with influenza (Vil'nyanskiy, 1941; Benevolenskiy, 1945); others, for example, Rubinshteyn and Mandel'shtamm (1940) deny this possibility. Our own material is insufficient to give us grounds for drawing conclusions on this question. However, keeping in mind the reduction in the body's resistance as well as the provoking effect of influenza on it and the deterioration in a number of chronic diseases under the influence of this infection we should allow the possibility of a harmful effect of influenza in the course of the tuberculous process. Our viewpoint is supported by the majority of authors occupied with this problem.

Certain authors (Krichevskiy and Shotter, 1927; Arsen'yev, 1938; Shakhazarov, 1939; Glukhov, 1949) describe signs of sepsis, pyemia, septicemia and metastastic abscesses as influenza complications. Schmiden (1919), Vogelsang (1928), Sommerford (1929), Nikulina (1938), Konschegg (1953) and others also mention them.

The majority of these observations were made before the discovery of the influenza virus, and should be considered doubtful in the light of the modern knowledge of its pathogenesis.

In our material we have not observed any septic, pye-

mic or septicemic processes as a result of having influenza.

In the literature, particularly that of previous years, there are indications of relapses observed in influenza (Leichtenstern, 1896; Kushev, 1932; Strupel, 1933; Bosse, 1935; Rubashova, 1935, and others).

The occurrence of relapses in various influenza outbreaks has also been noted by many practising physicians. We believe that these "relapses" represent cases of acute catarrh of the respiratory tract with which the convalescent has become ill or complications which were not revealed by the treating physician, which we have had an opportunity of observing repeatedly. The existence of true influenza relapses can be assumed only after it is confirmed by the laboratory.

Certain authors believe that influenza exists with a lingering course -- chronic influenza. Others deny the existence of chronic influenza. The diagnosis of "lingering influenza" has frequently been encountered in clinical circles in the comparatively recent past; at present, it is encountered only exceptionally.

The term "chronic influenza" was originated by the German clinician Franke (1930). Under the name of "Chronic Influenza" he wrote a book which came out in two editions, in 1900 and 1927, and which was translated into Russian in 1930. This diagnosis was taken up by a number of German authors (Kretz, 1906; Helpach, 1910; Armbruster, 1916; Treupel, 1920; Zülzer, 1925).

Certain Soviet authors also indicate lingering cases of influenza, for example, Yegorov (1929, 1930), Rozenberg (1934), Yal'tsev and Potekhin (1937), Dem'yanov (1939, 1946), and Dombrovskaya (1948).

Franke himself referred to the very great Russian clinical pediatrician, Filatov (1899) who assumed the existence of lingering influenza. Actually, Filatov described cases of chronic influenza in children as well as cases of chronic influenza in a physician in whose family there were three children who, in Filatov's opinion were also sick with chronic influenza.

Influenza is an acute disease in which the pathogen (virus) disappears quite rapidly from the respiratory tract. We have not observed any chronic influenza (virus) and cannot accept the existence of it. The lingering or chronic cases described in the literature are unconvincing with respect to the correct diagnosis of influenza. They were either diagnostic errors -- unrecognized cases of disease -- or complications of influenza produced by secondary infection, which assumed a lingering, chronic course. Among the latter, chronic catarrhal diseases of the respiratory tract, throat, ears, nasal sinuses were frequently encountered, particularly

in persons with labile heat regulation, sensitive to cold factors, in whom from time to time an exacerbation of the chronic diseases occurs.

69. Characteristics of the Clinical Course of Influenza and Its Variants

Sporadic and Pandemic Influenza. The clinical picture described applies to influenza which is observed during epidemic periods, during outbreaks of influenza in large centers. However, as has already been mentioned, influenza does not stop in the interepidemic period, being encountered in the form of isolated sporadic cases, sometimes as small local foci.

Sporadic influenza which occurs during the hot season of the year is distinguished from epidemic influenza by its less marked clinical picture, milder course and extreme difficulty in making the diagnosis. We noted this in our work in 1948, and it was confirmed by data on sporadic influenza worked out in conjunction with Fadeyeva in 1949-1950. This has also been noted by Kutsenog (1950).

As far as pandemic influenza is concerned, judging by the data in the literature it is no different from epidemic influenza.

At the present time, the spread of influenza in large centers is continuous throughout the year and produces outbreaks almost yearly, and sometimes twice a year, as occurred in 1946 and 1949. In such centers there are constantly large population groups which are immune to influenza, on the strength of which it usually does not reach an epidemic scale. This fact explains the comparatively favorable course of influenza, wherein severe forms of hemorrhagic pneumonias are a great rarity.

The favorable social conditions in which the Soviet masses are and which have been created in the Soviet Union because of the growth of technics, culture and public health cannot help but exert an influence on the spread and characteristic features of the clinical course of influenza. All this taken together considerably limits the spread of influenza and eliminates any particular severity in its course.

Atypical, Afebrile, Abortive, and Asymptomatic Influenza. Everything which we stated previously concerning the clinical characterization applies to the typical form of influenza which we observe most often during outbreaks of this disease. Along with this typical form of the clinical course of influenza cases of influenza may be observed which are distinguished from these "typical" forms in one respect or another. In atypical influenza various features

are usually absent which are characteristic of the typical clinical picture of influenza, or else a course which is not characteristic of it is noted. Some of the most pronounced signs of the toxic state including fever, which comprise the clinical basis of influenza, may be absent. When fever is absent we have the so-called "afebrile or non-febrile" form of influenza. Above, on page 345, the observations of Shimshelevich (1949) and of Stuart-Harris and co-authors (1938) have been presented. Influenza with a non-febrile course has been described by Leichtenstern and others. In material which we obtained in various outbreaks of typical influenza we noted a certain percentage of cases in which one symptom or another was absent which was important for making the diagnosis. Thus, in 5 to 17 percent of the patients there was no chill; in seven percent, no headache; in 10-40 percent there was no depression or rheumatic type pains; in 20 percent, no adynamia; no eye symptoms in 20-47 percent of the patients, etc. Sometimes, several features were absent simultaneously. Sometimes, an extremely mild, abortive course was observed in which the symptoms were hardly expressed. In such cases the diagnosis may be based only on the epidemic and laboratory data. Stuart-Harris and co-authors noted cases of influenza with hardly noticeable symptoms, when the entire disease lasted a total of only one day. Cases of influenza have occurred with such abortive and brief signs that they were difficult to observe and recognize as clinical symptoms of the disease. These are the so-called "inapparent," "asymptomatic" or "subclinical" cases observed by Shimshelevich (1949) as well as by Ritova (1951, 1953, 1954) in groups of younger children, and by Zhdanov and co-authors (1953), Orlov and Smil'gin (1945) in adult groups. Similar cases have been observed by Burnet (1947). These persons were in close contact with influenza patients. Despite the fact that they had no signs of the disease they all suffered from influenza. The increase in the influenza antibody titer which is observed in influenza may serve as proof of this. Consequently, they had influenza during an outbreak but this influenza did not express itself clinically.

Influenza in Persons Who Have Received Influenza Vaccinations. Recently, quite considerable groups of people have been vaccinated against influenza in many cities of the Soviet Union.

It is known that influenzal immunization reduces the influenza morbidity rate; however, it does not protect all those vaccinated completely, and a certain number of them become sick anyway.

Despite the tremendous number of works, both of Soviet and foreign authors, devoted to the study of the efficacy

of influenza vaccination, there are very few data concerning the clinical course of influenza in vaccinated persons. We managed to find indications of the course of influenza in vaccinated persons in the Soviet literature in the works of Dubakina and co-authors (1953) and Gukasyan (1955). Dubakina mentions that among the persons vaccinated against influenza a smaller percentage of cases of influenza has been noted with a severe course. Gukasyan vaccinated 3,516 persons against influenza intranasally in Leninakan in December 1953. Among those vaccinated there were 2.8 percent of the patients who had had influenza and acute catarrh of the respiratory tract; among those who were not vaccinated 8.5 percent of the patients had these diseases, that is, three times as many. Loss of work in those innoculated amounted to 4.9 days; in those who were not innoculated, 5.55 days, that is, 0.65 days more, which attests to the more favorable course of influenza in those vaccinated.

Among those vaccinated (Shimshelevich) the persons became sick 2.1-2.3 times less than among the unvaccinated, and the number of cases in which influenza had a mild course was higher. There were no serious cases in the vaccinated group. The duration of the febrile period among them was 0.49 days less; there was no temperature observed above 40°; the loss of work was 0.41 days less compared with the unvaccinated.

In the foreign literature there are few works on this problem. Bornemann (1952) writes: "in practice, immunization leads only to a lessening of the severity of the clinical course of the disease". In another work, Siegel and co-authors (1948) compares the course of influenza in 250 patients vaccinated three months before the disease with its course in 28 unvaccinated persons. In the former, the elevated temperature was found to be 0.9 days less, and the disease was 0.6 days shorter. However, the authors considered their results doubtful because of the incomparability of the figures (250 and 28). They explain the slight increase in antibody titer by the very high titer at the time of the disease owing to the recent vaccination.

There were 89 patients vaccinated against influenza under observation at the hospital of the Institute of Virology of the Academy of Medical Sciences USSR, and 81 unvaccinated patients. They came under observation from the very beginning of the disease, and the disease proceeded in them without any complications or associated diseases. It was shown that the signs of the toxic state (fever, dizziness, headache, depression and rheumatic pains, adynamia) were less pronounced in those vaccinated and lasted a shorter time; the catarrhal signs (coryza, cough) were less pronounced and disappeared more rapidly; the cardiovascular system

was less affected; leukopoiesis suffered less; leukopenia, eosinopenia and the shift of the neutrophils to the left were observed less often. The same thing was also noted with respect to the platelets. The duration of the disease and the amount of time lost from work were less in the vaccinated persons.

In the unvaccinated persons the number of complications were 1 1/2 times as much as in the vaccinated persons; the complications were more severe and longer lasting.

Fulminating Influenza. Along with the mild, abortive and asymptomatic courses a severe lightning-like course of influenza is observed with a fatal outcome during the first few days of the disease. Such very severe cases of influenza were described during the 1818/19 pandemic ("Spanish Flu"). Solitary cases are observed also during epidemic outbreaks of influenza, and from time to time also during interepidemic periods. The toxic state in the patients is marked, and there is an involvement of the cardiovascular system. Clinically, in fulminating influenza a severe headache, depression, sometimes temperature rise to 40° or more, cyanosis, dyspnea, marked adynamia, meningeal signs, rapid weak pulse, muffled heart sounds, marked hypotension, and sometimes vomiting are noted. In the majority of patients an unconscious condition is found, with delirium, but sometimes the state of consciousness may remain clear. Rales of different kinds may be heard in the lungs. Various catarrhal signs (rhinitis, pharyngitis, etc.) are observed. In children a pallor of the face, cyanosis and sometimes convulsions are noted.

The cyanosis, which appears before the pronounced involvement of the cardiovascular system, is explained apparently by the injury to the capillary and alveolar epithelium which leads to a decrease in the permeability for gases (oxygen) and to a reduction in pulmonary ventilation (Brauer).

At autopsy in fulminating influenza congestion and stasis are found in the brain matter and in meninges, congestion of the laryngeal mucosa of the trachea and bronchi, sometimes with superficial necroses, hemorrhagic necrotic laryngotracheobronchitis, congestion of the lungs with areas of hemorrhages in them, and sometimes small foci of dark red consolidation in the lungs, degeneration of the heart muscle, liver and kidneys. If death occurs on the second or third day, various numbers of hemorrhagic foci are found in various lobes of the lungs.

Influenza in Children. According to the data of Il'-yinskiy and Zverev (1935), Danilevich (1939), Zlatopol'skaya (1948), Maslov (1948) Olevskiy, Speranskiy (1950), Ritova

(1952), Filippova, Nutrikhina (1952), Maretskaya and others (1953), Ritova (1953), Ritova and Olevskiy (1954), Ritova and Stefanskaya (1954), Agababova (1954), in young children under six months of age and even up to one year of age the clinical course of influenza is different from that in children of older ages. In children under one year of age influenza is accompanied by signs of intoxication, whereby the toxic states has its own definite characteristics.

In suckling children the disease begins with loss of weight without any apparent cause. This situation lasts for three or four days. The temperature usually remains at a low-grade fever level. The child shows sluggishness and adynamia. The disease sometimes is accompanied by vomiting, convulsions, frequently diarrhea, and meningeal signs are also possible. Catarrhal signs -- rhinopharyngitis -- are less pronounced in them. In contrast to adults (puffiness and hyperemia of the face) the children are pale when they have influenza.

According to the observations of Koval' (1950), in young children influenza has a severe course: the normal development of the child is disturbed, and it loses weight. Frequently complications occur in the form of pneumonias and otitis. Similar descriptions have been presented in the works of Adams, Thigpen, Richard (1944, Marquezy and Debre (1950) as well as in the earlier works in Dub (1934), Dulitskiy and Karaseva (1934).

In children over a year of age the temperature reaction is expressed to a considerably greater degree than in children under one year of age. The toxic state is expressed in the same way but the catarrhal signs (nasopharyngitides) are less pronounced than in suckling children and particularly in adults, whereby the nasopharyngitides are usually of a purulent nature.

As far as children over four years of age are concerned, the clinical picture of influenza in them is, in general, the same as in adults (Bubnov, 1935; Varshevskiy, 1939; Rappoport, 1948, Cateigne and others, 1950; Ritova, 1951). In contrast to adults, influenza in children proceeds with a predominance of the sympathetic functions (Shimshelevich) over the parasympathetic functions of the vegetative nervous system.

This explains the pallor and diarrhea which so often occur in children and so rarely in adults. In children under one year of age influenza not uncommonly is accompanied by leukocytosis.

The question arises whether there is a difference in the clinical course of influenza produced by the pathogens A, A-prime, B, C and D, and whether it is possible to determine the nature of the virus producing the disease on the

basis of the clinical observation of the patients.

Certain works contain characterizations of the clinical course of influenza type A or type B (Nigg and co-authors, 1942; Stansfeld and Stuart-Harris, 1943; Bruce and Slavin, 1947; Shimshelevich and others, 1949; Tushinskiy and Korovin, 1949; Kofman, 1953; Stepanov and Zaytseva, 1953; Kutsenog, 1956). Certain authors (Shimshelevich, Nigg and co-authors and others) believe that influenza B has a somewhat milder course with a less pronounced toxic state and more marked catarrhal signs then influenza A. On the other hand, Leningrad authors (Korovin, Kofman) point out that in influenza B there are more complications (in the lungs and nervous system) than in influenza A. According to Kutsenog, influenza B has a more severe course than influenza A. Other authors could not establish the existence of any difference between these two types (Francis, 1940; Skryabina, 1953).

We have had the opportunity of observing all the outbreaks of influenza beginning with 1938. There were outbreaks of influenza A in 1938, 1939, 1943/44 and 1946; of influenza A-prime in 1949, 1952 and in 1953/54; and of influenza B in 1940, 1946/47, 1949/50, and a small outbreak of cases of influenza A and B in the winter of 1947/48. In 1947, we concluded that it was hard to distinguish the clinical picture of influenza A from influenza B, and on the basis of clinical observations it is absolutely impossible to make the etiologic diagnosis of the disease. It can only be stated that we are confronted with a case of influenza, but we are unable to determine which virus caused it clinically. Serological investigations of the patients have shown that influenza outbreaks may be of mixed nature. For example, in March-April 1949 during an outbreak of influenza A-prime there were 10 percent of influenza B cases, and at the end of 1949 and beginning of 1950 we had no less than 30 percent cases of influenza A and A-prime in a B influenza outbreak. The same thing has been noted also in subsequent outbreaks.

Although a general characterization of the epidemic give us an idea of the clinical course of influenza A or B, it is nevertheless better to analyze each patient individually rather than the epidemic as a whole, taking into consideration which influenza virus (A, B or C) caused the given case. Using this more exact analysis of the material it was shown that it is clinically impossible to distinguish influenza A and A-prime from influenza B.

For the purpose of a comparative characterization of the clinical manifestations of influenza, A, A-prime and B we are presenting data in Table 86 concerning the various signs of both types obtained in our hospital material in

1940, 1946/47 and 1949/50 (influenza B), in 1943/44 and 1946 (influenza A) and in 1949, 1952 and 1953/54 (influenza A-prime).

From Table 86 it is seen that in the various types of influenza there were signs of a toxic state and local catarrhal involvements of the mucosa, respiratory apparatus, pharynx, etc.

Table 86

Frequency of Various Symptoms of Influenza (in Percentages) During Various Epidemics[1]

Symptoms \ Type of influenza	B			A		A_1		
	1940	1946/47	1949/50	1943/44	1946	1949	1952	1953/54
Chill	83	87	92	90	88	96	88	95
Headache	100	98	98	98	100	93	98	100
Dizziness	25	62	44	45	31	51	48	30
Depression and Rheumatic Type Pains	70	68	72	70	60	75	81	90
Adynamia	84	96	80	93	96	98	97	100
Maximum temperature	39,6	40,3	40	40,2	39,9	40,8	40,6	40,6
Temperature less than 38°	22	18	15	24	27	9	20	5
» » 38,1 — 39°	59	52	57	53	57	49	43	49
Temperature over 39°	19	30	28	23	16	42	37	46
Temperature drop by crisis	24	15	24	23	26	10	23	88
Biphasic fever	25	15	11	7	15	10	18	6
Sweats	43	76	61	62	68	78	83	87
Herpes	14	22	17	24	19	25	10	16
Coryza and stuffiness of the nose	93	91	80	95	84	87	89	73
Nasal hemorrhage	2	8	4	3	4	14	8	5
Hyperemia of the Throat	87	90	—	75	85	69	62	58
Pains on Swallowing	40	28	26	27	13	26	24	28
Cough	90	96	84	94	90	93	80	79
Hoarseness	25	33	35	23	23	73	10	7
Pains in the chest	5	7	—	35	3	—	—	—
Bronchitis, tracheitis, laryngitis	77	83	63	93	70	83	—	38

Table 86 [continued]

Symptoms \ Type of influenza	B			A		A'		
	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Bradycardia	5	29	10	28	31	12	6	27
Tachycardia	15	10		2	3	6	3	8
Loss of appetite	10	29	40	27	21	67	51	37
Nausea, vomiting	1	6	4	7	2	6	10	13
Insomnia	8	10	13	9	3	15	13	15
Delirium	—	—	—	—	—	1	—	—
Neuralgia, neuromyositis	2	13	—	17	4	33	6	4
Pains in the eyes, conjunctivitis and others	53	64	65	74	59	80	60	68
Trace albuminuria	50	14	18	23	19	35	55	37
Microscopic hematuria	—	13	20	—	—	64	22	20
Pain in the ears	11	11	—	5	6	8	—	2
Leucocyte count of less than 6000	62	47	70	50	42	46	83	63
Leucocyte count of 6000–8000	27	43	21	40	30	42	17	21
Leucocyte count of over 8000	11	10	9	10	28	12	—	16
Sedimentation rate under 10 millimeters in an hour	87	44	67	60	40	60	53	41
Sedimentation rate of 11–15 millimeters in an hour	6	21	14	24	29	14	23	26
Sedimentation rate of more than 15 millimeters in an hour	7	35	19	16	31	26	24	33

[1] Our material on influenza C is inadequate for drawing the corresponding conclusion about it.

There is no or practically no quantitative difference (in the sense of frequency of occurrence of the symptoms) between the various sets of symptoms.

In our material suddenness of onset of the disease was observed in 67-86 percent of the patients in both influenza A and in influenza B; contact was mentioned by the patients in 23-48 percent of the cases, and the cold factor was mentioned in 8 to 33 percent. The average duration of the fever was from 3.6 to 4.3 days in influenza B; from 3.5 to 3.8, in influenza A, and from 3.6 to 4.6 days in influenza A-prime. The febrile period lasted one day in 6 to 12

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percent of the patients; two days, in 10-24 percent; three days, in 13-28 percent; four or more, in 42-70 percent in influenza B. The respective figures for influenza A are the following: one to four percent; 22-25 22-25 percent; 21-28 percent; 46-53 percent. For influenza A-prime: one to eight percent; 10-21 percent; 21-29 percent, and 42-68 percent. We observed complications in 25-58 percent of cases in influenza B; in 22-44 percent in influenza A; and in 24-42 percent in influenza A-prime. The percentage of complications in the respiratory tract, pharynx, nasal sinuses and ears was from 21 to 38 in influenza B; from 22 to 29 in influenza A; and from 20 to 42 in influenza A-prime. A mild course of the disease was noted in 10-56 percent in influenza B; in 25-39 percent in influenza A; and in 7 to 11 percent in influenza A-prime. A severe course was noted in two to seven percent of influenza B patients, three to four percent of the patients with influenza A, and 8 to 13 percent of the patients with influenza A-prime. The others had influenza of moderate severity.

The general condition of the body, particularly age, is of great importance in the severity of the clinical course of the disease and in the occurrence of complications. Thus, in 1940 (influenza B) 98 percent of our clinical patients were in the prime of life, 18-30 years of age, and of these 56 percent had mild influenza and only two percent, severe influenza. Complications were observed in 28 percent of the patients. In 1946/47 (also influenza B) with 29 percent of the patients from 18-30 years of age a mild course of the disease was observed in 21 percent of the patients, and complications occurred in 58 percent. Approximately the same thing could be observed also in other influenza outbreaks (A, A-prime).

Our observations give us the grounds for the belief that the difference in the epidemics depends not so much on the type of virus as on the condition of the persons who are having the infection. It is important to note the relationship between the age of the patients and the nature of the course of the disease.

Clinical Aspects of Influenza Type C. The clinical picture of influenza C is reflected in a quite scanty manner in the literature (Francis and co-authors (1950) and Gerber and co-authors (1952) from the United States; Farkas, Dömök, (1954) -- Hungary; Styk, 1955 -- Czechoslovakia; Ritova and others). All these authors agree that influenza C resembles mild influenza with a mild toxic state in its course. It occupies an intermediate position between typical influenza and ordinary acute catarrh of the respiratory tract. In influenza C the catarrhal signs are more pronounced than influenza A, A-prime and B. The younger the patient

the more pronounced the catarrhal signs in the nasal mucosa (coryza), pharynx (acute pharyngitis), tonsils (tonsillitis), and sometimes bronchi (cough). In influenza C bronchitis, pneumonia as well as otitis are noted. Conjunctivitis observed during an outbreak of influenza C in nurseries in Bratislava (Czechoslovakia) in 1952 attracts attention. The conjunctivitis usually disappeared in several days, but sometimes dragged out for several weeks or more (Styk).

If there was a fever it was low (under 38°) and brief; in addition, dizziness, nausea and pains in the eyes were observed. The disease sometimes is diagnosed influenza (mild), but more often it is called an acute disease of the respiratory tract. Data obtained by Quilligan, Minuse, Francis and Arbor (1954) in experimental infection of volunteers with the influenza C virus are interesting. After intranasal infection the latter did not become sick. Instead, on the seventh day after the experiment the experimenter became sick. He developed a rhinitis with a slight discharge from the nasal cavity. The next day, these signs increased, and cough was added. On the third day, malaise, pains in the eyes, and sneezing occurred. The coryza was moderate. Temperature remained normal all the time. On the fourth and fifth day, the condition remained unchanged. On the sixth day, the pathological signs mentioned disappeared, but adynamia remained for a certain time. The antibody titer against the C virus in the patient's serum increased bu four times from the second to the 12th day of the disease.

Therefore, it was shown that the incubation period in influenza C is longer than in cases produced by other types of influenza virus. Catarrhal signs predominated in the picture of the disease.

In giving a general summary of the data in the literature concerning the clinical course of influenza produced by virus C it may be noted that it is encountered among cases of influenza produced by other types of virus (A-prime, B). It is distinguished by a longer incubation period and is frequently observed among children and adolescents. Influenza type C has a milder course. It frequently occurs without any pronounced toxic state and is accompanied by low-grade or normal temperature as well as by pronounced and predominant catarrhal signs in the respiratory tract and conjunctiva, particularly among children. In the severity of its clinical course influenza C occupies an intermediate position between influenza and acute catarrhs of the respiratory tract. According to our data, in adults the clinical picture of influenza C is not much different from that of influenza A, A-prime, and B. As far as influenza type D

is concerned a widespread outbreak of this disease was observed among adults (Bladivostok); among children (Moskovskaya Oblast') it was distinguished by a severe course. All this may be explained by the low level of immunity in the population against this virus (Gerngross, 1957; Zhdanov, Ritova and Golygina, 1957).

Prognosis. Influenza lasts several days and usually culminates favorably, particularly in young and healthy people who do not have any chronic pulmonary diseases, etc. Uncomplicated cases turn out well as a rule. The prognosis is more serious in young children, particularly in sucklings, in weakened persons, elderly persons, and in patients with chronic diseases, and in patients with complications, particularly in the lungs. Cases of primary influenzal virus hemorrhagic pneumonias have a poor prognosis and usually terminate fatally. Four such patients who entered our hospital died in the first few days of the disease. In other forms of influenza we have not observed any fatal outcomes, despite the considerable number of complicated cases, including complications of secondary pneumonia (microbial origin). Possibly this is explained by the fact that our observations for the most part pertained to the people in the prime of life and that we made extensive use of sulfonamides and antibiotics.

70. Classification of Clinical Forms of Influenza

Various influenza classifications are known. In the first half of the 18th century, and specifically in the epidemic of 1743, Hexham in Scotland noted the following forms of influenza: encephalica, thoracica and abdominalis (according to Kuznetsov and German, 1890), that is, neural, respiratory and gastrointestinal. In the pandemic of 1889/90, particularly in 1918/19 and subsequently, various classifications of influenza were proposed. Mention was made of a catarrhal form (with subdivision of it into a group with involvement of the upper respiratory tract and with involvement of the bronchi and bronchioles), inflammatory, neural with involvement of the central and peripheral nervous systems, respiratory, gastrointestinal (with catarrhal and ulcerative lesions), pulmonary (with various forms of pneumonias), rheumatic, typhoid, cardiac, etc.) (classification of Heine and Ekk (1852), Vasil'yev (1889), Leichtenstern (1896), Afanas'yev and Vaks (1904), Troitskiy (1919), Glinchikov (1922), Chistovich, Sadov (1927), Lezhnev (1932), Strümpel and Sticker (1933), Rozenberg (1934), Braude (1939), Barkhash (1939), Ionin (1942), Rozen'yer and Sizemova (1948), Tushinskiy (1951) and others).

All the classifications listed are without any viro-

logical basis. They do not contribute to a better diagnosis of influenza; on the other hand, they give us the grounds for distinguishing special forms of influenza, namely: gastrointestinal, rheumatic, typhoid, dysenteric, encephalitic, and lead to a large number of diagnostic errors.

Based on these considerations, we believe that Konchalovskiy (1927) was right when he suggested dividing influenza into uncomplicated and complicated forms. Rossiyskiy (1945, 1949, 1951) adheres to the same viewpoint. We also recommend speaking about uncomplicated and complicated influenza. As far as complications are concerned, they can be observed in various organs and systems; in addition, exacerbations of various chronic diseases from which the patient suffered before having influenza may be observed.

Gal'perin (1953) divides the forms of influenza according to the severity of the clinical course of the disease. Here, he keeps in mind the general condition of the patient, the evaluation of the condition of the body as a whole rather than of various organs or systems. The author divides influenza into the following forms: abortive, mild, of moderate severity, severe (toxic) and hypertoxic (very severe). He makes an attempt to give objective characteristics by which it might be possible to define the severity of the disease.

Beginning with 1938 we have noted "pure" influenza in the hospital, that is, influenza without complications or associated diseases, and complicated influenza with associated diseases or without them, with a mild, severe or moderate form of course. Such a division is adequate from the point of view of defining the severity of the course of the disease. However, such a characterization does not give us a complete idea of all the forms of clinical manifestations of influenza, which is important in a practical respect.

For the purpose of including all the clinical forms of influenza and taking account of the severity of the course we are suggesting the following classification of influenza (Table 87).

Usually (80 percent or more) cases are observed in which the main signs of influenza are present: signs of a toxic state (fever, headache, adynamia, and others), in varying degrees of severity, and catarrhal signs of the respiratory tract (coryza, cough, etc) occurring from the beginning of the disease or after a certain delay. This form is usually "typical" of influenza and can occur without complications or give rise to complications in various organs and systems.

Table 87

Classification of the Clinical Forms of Influenza and Their Course

A. According to Clinical Manifestations	B. According to Severity of the Clinical Course	
I. Typical Influenza (with toxic state and cattarrhal signs)	1. Uncomplicated	a) Mild, with slight toxic state
		b) Moderate, with moderate toxic state
		c) Severe, with very pronounced toxic state
	2. Complicated	Usually, of moderate severity and sever. A mild course is also observed
II. Atypical		
a) Non-febrile, afebrile	1. Uncomplicated	a) Mild
		b) Moderate
	2. Complicated	c) Severe
b) Non-catarrhal (without noticeable catarrhal signs)	1. Uncomplicated	a) Mild
		b) Moderate
	2. Complicated	c) Severe
c) Fulminating		
1. With the development of hemmorrhagic pneumonia ("pulmonary" influenza, "Spanish 'flu" of the pathologists)		Very severe, with marked toxic state (usually fatal)
2. Without clinically noted hemorrhagic pneumonia		Very acute course with marked toxic state and quick death prior to formation of hemorrhagic pneumonia

The severity of the clinical course of this type of influenza is different, depending on the degree of expression of the toxic state, and in part, of the catarrhal signs. The disease may occur in the mild form with a mild toxic state. Such patients usually remain on their feet and, being in contact with healthy persons, infect those around them. The disease may be of moderate severity, where the toxic state is expressed to a moderate degree, and the patient must lie down. The disease can also assume a severe character with a very pronounced toxic state, sometimes with

prostration, marked apathy, sleepiness, less often with excitation and delir rium.

An evaluation of the severity of the clinical course of influenza (toxic) cannot be given on the basis of the degree of expression of any one symptom. The temperature, for example, is not of any decisive importance. Cases are observed in which a considerable temperature reaction (38-39° or more) is present with a relatively satisfactory condition and feeling of well-being, with a comparatively mild course of the disease. On the other hand, a low temperature (less than 38°) may be observed with a poor feeling of well-being and a serious condition, sometimes with prostration. Now, it is still impossible to make any objective criteria for evaluating the various subjective complaints in order to decide the severity of the course of influenza. A determination of the severity of the clinical course of influenza may be made only on the basis of all the subjective signs (headache, depression, adynamia and others) and objective data (temperature, condition of the cardiovascular and nervous systems, etc.) taken together.

The signs of the toxic state determine the severity of the clinical course of influenza. The catarrhal signs have relatively little influence on the general condition of the patient, although marked catarrhal signs and particularly complications in the respiratory organs may exert a harmful effect on the body.

In addition to the usual, most widespread typical form of influenza indicated, atypical forms are observed in which various cardinal symptoms are absent, for example, fever, catarrhal signs, or in which influenza is expressed in the fulminating form, which is of unusual severity.

An afebrile type of influenza is observed (Glinchikov, 1932), that is, cases in which the disease proceeds without a temperature reaction. Such cases also are observed by practicing physicians, and the existence of them should be known. Influenza of this form may take on different clinical courses -- from mild to severe. It can occur without complications but may produce various complications.

The clinical diagnosis of afebrile influenza is not difficult during an outbreak; in the interepidemic period such cases may offer great diagnostic difficulties, and their diagnosis is confirmed by the laboratory.

Another atypical form of influenza is observed -- the "non-catarrhal," where influenza proceeds only with the signs of a toxic state and with a complete absence of catarrhal signs (coryza, cough, etc.). Such cases have been observed, for example, in our hospital material during an influenza outbreak in 1952 in four percent of the cases; in December-

January 1953/54, in 14 percent.

The non-catarrhal cases of influenza may also assume a milder or more severe course, may occur without complications or may produce various complications.

Recently, statements have begun to appear (Burnet, 1947; Zhdanov, 1950; Ritova, 1951; Orlova, 1954) that there are subclinical, asymptomatic or inapparent forms of influenza where persons in the vicinity of influenza patients can note the increase in antibody titer against various types of influenza virus (evidence of having the disease) without clear-cut or detected clincial signs of the disease. Such cases may be determined only by the laboratory route. There are also abortive forms in which the main symptoms are very slight and the disease assumes an exceptionally mild course, and the patient usually does not stop his work. Such influenza may be diagnosed only by the laboratory but not clinically; therefore, they cannot be spoken of as a special "clinical" form of the disease in the almost complete absence of a clinical picture. We are not including these cases in the "forms of clinical course of influenza," because they may be of epidemiological but not necessarily of clinical significance.

Among the atypical forms of influenza are also the fulminating cases, namely: 1) "pulmonary" form and 2) the fulminating form proper.

"The pulmonary" form or primary (hemorrhagic) influenzal pneumonia (Spanish 'flu") of the pathologists occurs when the local virus involvement is not limited by the mucous membrane of the upper segment of the respiratory tract but rather is concentrated in the bronchioles and in the pulmonary parenchyma. Then, a specific virus pneumonia is created which is produced by the influenza virus.

The fulminating type of influenza proper occurs in an extremely acute and severe form accompanied by a marked toxic state, and a condition like collapse. Such influenza rapidly (during the first two days) leads to a fatal outcome, sometimes before hemorrhagic pneumonic foci can be created. The fulminating form of influenza, unfortunately, is not frequently observed; nevertheless, it is noted in a variable number of persons during any epidemic (it was encountered with particularly great frequency in the pandemic of 1818/19), as well as during interepidemic periods from time to time. It is usually encountered among children of the younger age group and more frequently the younger the child (in children under three years and particularly among suckling children).

The typical and the afebrile and non-catarrhal types of influenza may occur without complications or with complications, with associated diseases or without them, in a

mild, severe form, or in a form of moderate severity. Fulminating influenza occurs in a very severe form, and death usually occurs before the development of any clinically detectable complications.

The classification which we have proposed is not based on localistic, organic lesions ("gastrointestinal," "pulmonary," with its various types of involvement, "rheumatic" type of influenza, etc.), but rather characterizes the reaction of the body as a whole to the incorporated infection and the degree of expression or the absence of the main signs of the disease. This classification gives us an idea about each individual case, for example, typical influenza, uncomplicated, mild or typical, complicated, severe (complication -- focal pneumonia of the upper lobe of the right and lower lobe of the left lung), or afebrile, uncomplicated, moderate influenza, or non-catarrhal, complicated, moderate influenza (complication -- pyelitis), or atypical fulminating influenza, etc.

It goes without saying that we should not let the associated diseases be overlooked (acute or chronic); these may make their impression on the clinical course of influenza and on the general condition of the patient. In characterizing the case they should not be allowed to slip out of sight; they should be pointed out, for example: uncomplicated case of influenza of moderate severity with associated chronic left-sided purulent otitis or non-catarrhal, moderate case complicated by pyelitis with associated chronic cholecystitis, etc.

The classifications suggested should contribute to a better diagnosis of influenza and more effective control of it.

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SEVENTH CHAPTER

EPIDEMIOLOGY

71. General Information on the Epidemiology of Influenza Before 1933

The time of occurrence of the first influenza epidemics, as has already been mentioned in the first chapter, cannot be established. Afanas'yev and Vaks (1904) point out that some investigators consider that diseases described in 412 B.C. by Hippocrates and Livy as well as epidemics of epidemic cough in the fifth to tenth centuries which were called epidemics of "Italian fever," were influenza. However, the authors doubt the influenzal nature of these diseases and consider 1173 the date of the first influenza epidemic. At that time, this disease had extended into Italy, Germany and England.

A list of 90 epidemics which occurred from 1173 through 1875 has been given by Hirsch (1881), who made an attempt to distinguish influenza from simple epidemic catarrh, but collected absolutely heterodynamic data, including both small local outbreaks and large-scale epidemics and pandemics in his list.

More exact data have been given by Mote (1943), who arranged the known influenza epidemics in a definite chronological order (Table 88).

Table 88

The Most Important Influenza Epidemics

Years	Distribution
1510	First European epidemic
1557	Second European epidemic
1580	Third European epidemic
1593	Fourth European epidemic
1647	First American epidemic
1655-1658	Epidemics which began in America and spread to Europe

Table 88 [continued]

Years	Distribution
1675	Epidemics in England and France
1698	Epidemics limited to North America
1709-1712	Large-scale epidemics in the countries of the Eastern Hemisphere
1729-1733	World pandemic spreading from the East to the West
1757-1762	Epidemics which began in North America and spread to South America and Europe
1767	Epidemics in America and Europe
1772	Localized epidemics
1775-1776	Epidemics limited to England and part of Europe
1780-1782	Pandemics which began in North America and which extended to Europe and Russia (1782)
1788-1790	Pandemics which began in Prussia and which spread to the West
1798-1803	Series of epidemics which began in North America and which spread to the East
1830-1833	Pandemics which began in China and which spread to the West
1836-1837	Pandemics which began in Russia and which spread to the South and West
1847-1850	Pandemics of undetermined origin
1857-1858	Epidemics noted in countries of both hemispheres
1873-1875	Epidemics in the countries of the Western Hemisphere
1889-1895	World pandemic which spread from the East to the West
1918-1919	World pandemic which began in North America

The most reliable dates of mass spread of influenza are given in this list of influenza epidemics. Dobreytser (1935) gives almost the same list. Here the terms "epidemic" and "pandemic" have been used arbitrarily, because they do not reflect the true situation owing to the lack of precisely recorded statistical material.

According to Francis (1953), the first influenza pandemic occurred in 1580, and the first epidemic in America, in 1758.

Vasil'yev (1889) counts 80 influenza epidemics from 1173 through the year in which he wrote his work, of which 57 were in Russia.

Indisputable dates of pandemic spread of influenza are 1889 and 1918. It can hardly be considered that the sites of origin of the epidemics can be seriously discussed. In this respect the pandemic of 1918 is an exception; its

onset was marked by outbreaks of cases which developed among military units who came from the United States to France during the First World War.

With respect to the site of occurrence of the pandemic of 1889 it cannot be considered that Leichtenstern's and Sticker's (1912) opinion is sound; they believe that it began in May in Turkestan, from where it was imported into Russia and Persia in the summer.

Gamaleya's (1942) statement to the effect that there was an epidemic in Greenland and on the territory of Hudson Bay in the spring of 1889 and that in May it reached Bokhara, remaining there until August, "then spread across Siberia and reached St. Petersburg in October" is evidence of the confusion of ideas existing on this subject. The territories listed are located so far from one another that with the means of transportation which existed in 1889 this route of spread of the epidemic is not very probable. It is possible that in this case there were influenza outbreaks unrelated to one another or historically unreliable information of a purely suppositional nature or outbreaks of infectious diseases erroneously assumed to be influenza.

Nevertheless, the 1889 pandemic is of exceptional interest, because beginning with the end of the nineteenth century an essential change occurred in the character of the epidemic process, which assumed a form approaching modern influenza, as will be explained below.

It is known that minor outbreaks of influenza were recorded in October 1889 in St. Petersburg, Warsaw, Berlin, Stockholm and Paris. In November and December, an epidemic spread of influenza was noted also in other cities of Russia and Europe. In the middle of December an epidemic broke out in Madrid, Lisbon, Belgrade and Verona; at the end of December, in Corsica, Algiers, Tunis and New York.

In 1890, influenza spread everywhere: in January it broke out in Egypt and South America; in February, it reached Japan and Hongkong; in July, Senegal; in August, the island of Jamaica, the island of Mauritius and the island of St. Helena. China was subjected to influenza in September; in October, influenza penetrated into Central Africa, and in November, into Abyssinia.

In France an epidemic began in Paris; then it went into the cities of the provinces, and from there to the villages. The spread of influenza was so rapid that in two weeks (from 16 December to the first few days in January) almost the entire country had been included in the epidemic. According to Lavergne and Dopter (1932), 40,000 persons died from influenza and its complications in France at the time of the epidemic.

In Germany, according to Friedrich (1894), the

morbidity rate in various cities was from 146 to 710 persons per 1000.

Sperling (1892) collected the mortality rate statistics for influenza in 200 German cities having a population of 11,500,000 persons, which at that time constituted about one-fourth of the population of Germany. In the year of influenza, 1890, the total mortality rate of the population rose by 1.2 percent compared with the average mortality rate for the preceding three years. According to his calculations, in 1889/90, 66,300 persons died from influenza in Germany, and 18,800 persons less than anticipated were born.

In the United States (Gamaleya, 1942) 340,000 of the population of 850,000 in Massachussetts became sick with the disease.

The dynamics of spread of influenza in a large city are shown in Fig. 176, where data are presented of the daily recording of new cases in Munich in December-January 1889/90. As is seen, the increase in the morbidity rate began in the middle of December and reached a maximum during the first 10 days of January. The duration of the outbreak was 45 days.

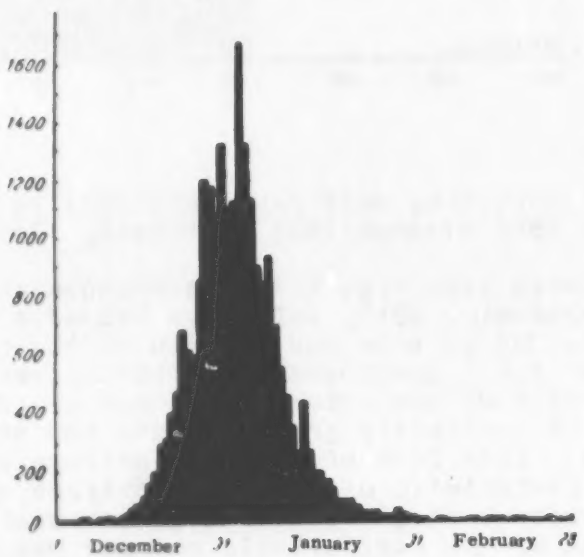

Fig. 176. Dynamics of Influenza Morbidity Rate in Munich in 1889/90 (Mote, 1943).

In all, the 1889 pandemic lasted about three years and left a generally increased influenza morbidity rate in its wake, which is shown by the statistical information on the increasing influenza mortality rate.

In this connection, the data of English statistics, presented in Fig. 177, are interesting.

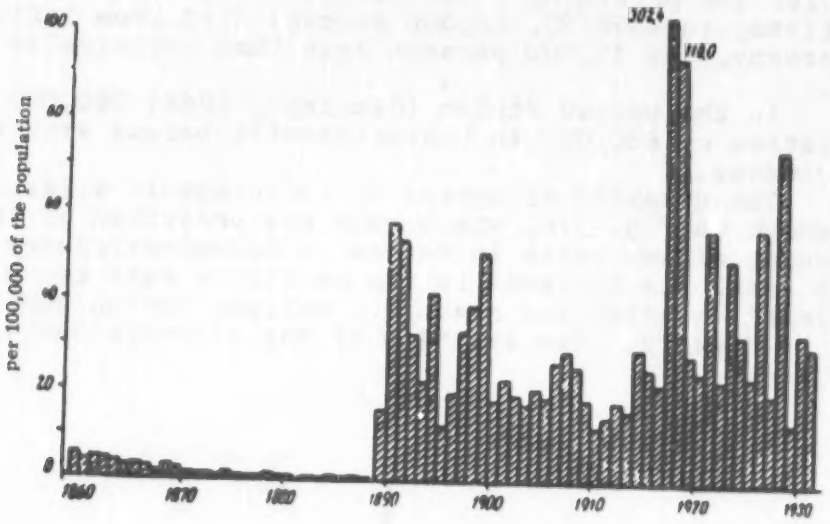

Fig. 177. Mortality Rate from Influenza in England from 1860 through 1933 (Andrewes, 1954).

As seen from Fig. 177, beginning with 1890 and until the next pandemic, 1918, influenza caused a mortality of from 120 to 588 persons per million of the population. This speaks for a considerable morbidity rate as well as for the fact that the epidemic process assumed the form of an infection constantly present among the urban population of England. This form of epidemic process afterwards became characteristic of all the countries with a temperate climate, because at the end of the nineteenth century the development of the capitalistic economy was completed, accompanied by the spread of railroads, the appearance of mechanized water transportation, the extension of international trade and the growth of the population in cities through the creation of large industrial centers. All this changed the form of communication between people and had a decisive influence on the spread of the influenzal infection.

It should be noted that in the foreign literature this very interesting phenomenon has not only not been given any acceptable explanations but, conversely, it has been made a kind of mystery. Thus, Andrewes (1954) writes that the facts which underlie the "dramatic" change in the nature of the epidemic process after 1889 are at present "a mystery."

This form of epidemic process, which from time to time has produced an increase in the morbidity rate in various countries, localized in its distribution, underwent a marked change at the end of the First World War. We must agree with Gromashevskiy (1947), who believes that the subsequent 1918-1920 pandemic occurred in connection with the prolonged disturbance in the normal communication between countries produced by the stable fronts of the predominantly positional warfare in Europe. As a result of this, a separation of large population masses occurred for a long time, and within these masses the sources of infection were able to exhaust themselves completely.

According to Frost's data (1919), localized epidemic outbreaks of influenza appeared in January 1918 in New York and other cities of the United States located on the coast of the Atlantic Ocean. They occurred among the civilian population and particularly in army training camps, naval bases and among military units which were being shipped to France. In April the influenza epidemic included not only the Eastern but also the Central and Western States of the United States.

It is natural, therefore, that the first European country involved in the pandemic should be France, where the units of the A.E.F. were being sent regularly. According to Lavergne and Dopter, influenza appeared in the French Army in April of 1918, and at the end of May there were no less than 25,400 cases. The epidemic soon spread throughout all the armies, involving, however, the infantry units predominantly and not so much affecting the more isolated artillery units.

It should be pointed out that this forst wave of the pandemic was distinguished by a benign course in its clinical manifestations. In June the morbidity rate decreased to 12,300, and in July to 2950. Fatal outcomes were recorded in May (24 cases), in June (14) and in July (6). To the same extent, influenza spread throughout the units of the British Army located in France and among the civilian population with a similar reduction in the morbidity rate in July and August.

The first 1918 pandemic wave spread rapidly to other countries in Europe; at the end of May and beginning of June influenza appeared in England, Spain and Portugal;

then, in Germany, Greece, Serbia, Poland, Rumania, Norway, Sweden, Holland, Belgium, Denmark and Switzerland.

In North Africa the influenza epidemic was first noted in Tunis after the arrival of a steamer with passengers from Europe and later in Egypt. In India, the first outbreak appeared in Bombay and then in Calcutta and Madras. In the Far East, particularly in China, the outbreaks of influenza were noted beginning with May and June. The latter constituted the basis for the unfounded assertion that the influenza 1918 pandemic started in China and later moved from the East to the West (Dujaric de la Rivière, 1932).

In short, at the end of the first half year of 1918 influenza had spread throughout the entire world, with the exception of Australia, where a repeated quarantine had been imposed on all passengers arriving on ships. True, the quarantine measures did not prevent but rather only postponed the occurrence of the epidemic in Australia, where it broke out only in 1919, shortly after the arrival of transport ships with demobilized soldiers.

After the decrease in the morbidity rate in July and August 1918 influenza appeared again in countries which had suffered from the first pandemic wave and in countries which had been left untouched until then. The second wave was different from the first in the more severe course of influenza, the frequent complications in the form of pneumonias and the increased mortality rate. It included the entire American continent, Africa, Asia and Europe.

While in September the mortality rate in the United States reached 10-15 persons per 1000 population, in October-November it rose to 60-65 persons. In England during the fall-winter rise in morbidity rate 220,000 deaths were recorded in total, and in Italy, 270,000. In South Africa the morbidity rate ranged from 321 to 460 persons per 1000.

In France at the end of August the epidemic spread to the military units both in the rear and at the front, particularly to the artillery units which had been only slightly affected by it in the spring outbreak.

In August, 3153 cases were recorded and 243 cases of death, whereas in July there were a total of 6 deaths per 2950 cases.

In September the epidemic increased, and 2124 deaths were noted in 24,282 cases. The first cold weather contributed to the occurrence of complicated forms of influenza, which reflected on the increase in the mortality rate: there were 16,017 deaths per 75,719 cases in October.

The epidemic began to wane at the beginning of November, and in December there were 16,383 cases (Fig. 178).

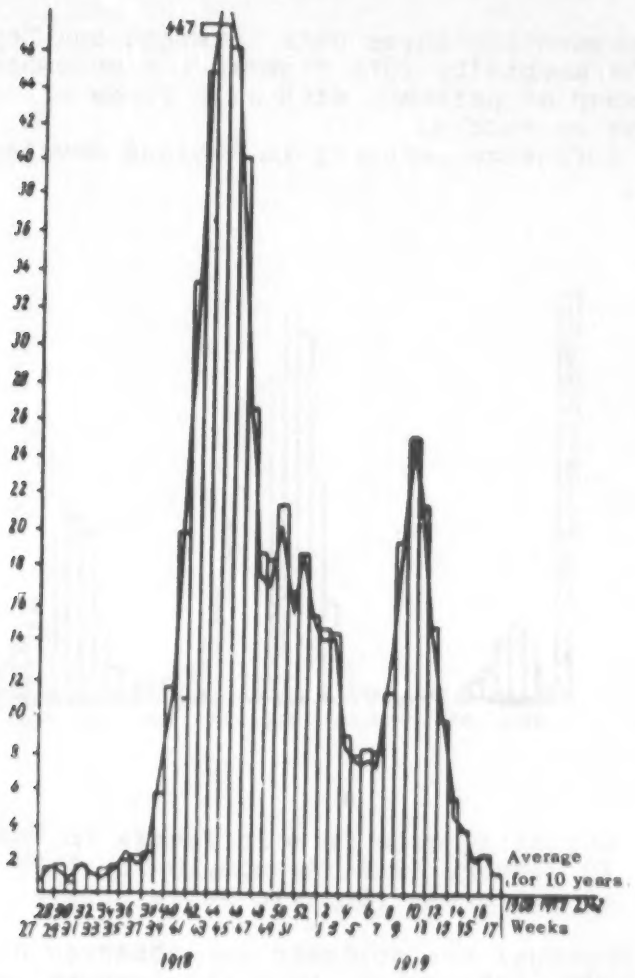

Fig. 178. The Mortality Rate from Influenza (per 100 Deaths) in Paris Between the 27th Week of 1918 and the 17th Week of 1919.

The columns represent those who died in Paris; the continuous line, the number of inhabitants of Paris (Dujaric de la Rivière, 1932).

In sum, influenza affected 408,180 persons from the beginning of April to the end of December in the entire

French Army (front and rear), that is, 126 persons per 1000, with a mortality rate of 30,382 persons, that is, 9.38 per 1000.

In presenting these data Lavergne and Dopter point out that the morbidity rate figures are undoubtedly too low. A large number of patients with mild forms of influenza were not put on record.

The influenza pandemic in England developed similarly (Fig. 179).

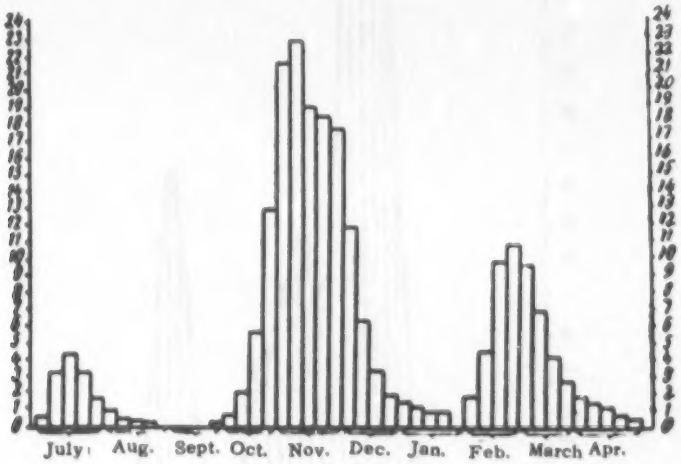

Fig. 179. Mortality Rate from Influenza in England from 19 January 1919 Through 10 May 1919. Three Waves of Influenza (Dujaric de la Rivière, 1932).

In Portugal the epidemic was observed chiefly during the first half of November, when the largest number of fatal cases was recorded. In December, the morbidity rate dropped notably. It should be noted that the Azores Archipelago, which had escaped the spring epidemic, suffered seriously during the second wave, after the arrival of an American ship on 21 September; in December, the maximum of the epidemic outbreak was observed.

In Sweden, Holland, Denmark and Germany the maximum morbidity rate was noted in October 1918.

In South America the epidemic wave spread toward Brazil, where it broke out in Rio de Janeiro at the end of September, reached a maximum on 20 October. In the second half of the same month it began in San Paulo and Montevideo,

reaching a maximum on 7 November, and terminated at the end of December. Bolivia remained untouched, and Ecuador suffered in December.

In Africa, which was seriously involved by the second pandemic wave, influenza did not stop, and in Egypt the maximum morbidity rate continued into December and January. The epidemic ended only in February, after causing 153,880 deaths. In India the epidemic of severe influenza spread throughout the country, beginning in September. In Bombay and Madras the highest morbidity rate was noted in October; in Calcutta, in November. The epidemic ended in December. The mortality rate was very high and was reckoned as 7,000,000.

In the Philippines the epidemic began in October and lasted until March 1919. The number of fatal cases reached 85,000 in the population of 10,000,000.

The third wave of the pandemic was customarily considered the disease observed beginning with January 1919. In this last outbreak influenza spread to a much lesser degree than in the preceding epidemics. The third wave spread throughout almost all of Europe and ended in May.

In America, chiefly in Uruguay, the epidemic returned later: it began in July 1919 and ended in August. In this country, as in all the countries of the South American continent, only the last two waves occurred. In Egypt there was no third epidemic period at all.

Madagascar, to which the epidemic had not spread previously, suffered severely from the 1919 outbreak. The infection was brought here by a naval transport vessel from Europe.

In India outbreaks occurred which were not of an extensive nature, with the exception of the province of Madras; in the other places the morbidity rate was limited to multiple isolated foci in inhabited places, without extending to villages. The same thing was noted also in the Philippines. Let us recall that Australia was included only in the third wave. Beginning there in January 1919, it produced a monthly increase in the number of cases up to June, after which it began to fall off rapidly.

We do not have at our disposal any precise information concerning the morbidity rate in Russia during the pandemic of 1918-1919. There are a number of interesting and valuable works written by Russian physicians and investigators, but they are devoted chiefly to problems of the clinical aspects and pathology. No regular information was collected on the spread of the epidemic, because, as is known, 1918 and 1919 constituted an entire era in the political and governmental life of the country. During these years the young Soviet Republic surmounted hunger and

economic collapse, defended the country against interventionists and fought a civil war. Nevertheless, the existing statistical information on certain cities permit us to judge that the complexified social conditions not only did not lead to a higher morbidity rate compared with foreign countries, but rather contributed to a decrease in it. The indices of influenza mortality rate per 100,000 population (Tables 89 and 90) presented below according to Dobreytser (1936) constitute evidence of this.

Table 89

Mortality Rate From Influenza in Foreign Countries

Year	England	Germany	Holland	Denmark	Spain	Norway	Switzer-land	Sweden
1918	336	293	258	336	710	286	543	471
1919	122	68	23	138	101	67		196

Table 90

Mortality Rate From Influenza in Russian Cities

Year	Moscow	Petrograd	Odessa	Saratov
1918	25,7	94,2	100,7	67,3
1919	39,9	57,9	66,0	33,7

The mortality rate figures in the United States should be added to this information; in 1918, they amounted to 298 per 100,000 population in the cities; 301, in the rural localities.

From a comparison of the data presented it is seen that the maximum mortality rate which occurred in Odessa in 1918 was equivalent to the minimum figure for mortality rate in the foreign countries during the height of the pandemic. In other cities the mortality rate from influenza was even less. Hence, it may be concluded that the morbidity

rate in the Soviet Union could not have been higher than in the other countries of the world, and that it was distinguished by a lack of uniformity in its territorial distribution.

The total mortality rate in all the countries during the influenza pandemic, according to the calculations of Jordan (1927), amounted to 20,000,000 persons.

In analyzing the material of the morbidity rate and mortality rate during the pandemic, it should be noted that the division of it into three phases or three waves which was done by all authors can be considered arbitrary. Between these waves the morbidity did not cease but only lessened, and in the countries of the temperate zone this decrease was associated, as a rule, with the hot part of the year, or more exactly, it was most apparent in July and August. There have been repeated attempts to explain the qualitative change in the course of the disease, which expressed itself in an increase in the mortality rate among the patients during the second half of 1918 and in 1919 by the existence of a special "pandemic" virus. Even modern authors have made attempts of this kind. For example, Francis (1953) believes that the influenza pandemic of 1918 was produced by a highly virulent virus. He believes that this virus existed even prior to the pandemic but was avirulent and therefore did not give rise to a high mortality rate. Later, he mentions that the influenza virus possesses a "limited set of components," the combination of which produces various strains. Andrewes (1954) considers it probable that in the port cities of Western Europe where the American troops arrived a mixture of different races of people occurred and that the new hybrid virus with malignant properties was created, so to speak, on this soil. An unconvincing reference to the data of Burnet's works on genic recombinations of influenza viruses serves as the only confirmation of this supposition.

In addition, Andrewes assumes the occurrence during the pandemic of "several virus mutants with different antigenic properties but with the common power to kill young persons."

The factual data, however, do not permit us to share any of the suggestions listed. As a matter of fact, the presence of "malignant influenza," or, in other words, of hemorrhagic pneumonias as well as purulent bronchitides, pleurisies and bronchopneumonias leading to a rapidly fatal outcome, was described even at the beginning of the pandemic. The quantitative increase in these forms in the later development of the pandemic can rather be connected with the general increase in the number of cases. The highest mortality rate noted among young persons permits us to con-

sider this attributable to secondary complications of bacterial microflora of which there was more in the air of rooms the greater the density of people in them. Barracks and other quarters were overfilled with service men, among whom young persons predominated.

It would not be amiss to recall that in 1918 the number of persons in the Entente armies on the Western Front alone amounted to 3,500,000 and that a more or less accurate account of the influenza mortality rate was kept among the troop contingents.

The facts of artificial infection of people by means of the introduction of filtered and unfiltered gargle washings, of sputum, mucus, lung tissue taken from influenza patients who died from it in 1918-1919 also speak against any special malignant pandemic virus. This research was carried out by Zelter (1918), Nuzum, Pilot, Stange and Bonar (1919), Yamanouchi, Sakakumi, Iwashima (1919), McCoy and Richey (1919), Leake (1919), Lister and Taylor (1919), Wahl, White, and Lyall (1919), and Schmidt (1920).

In all, the investigators listed infected 263 persons, of which 25 fell ill with influenza. A severe course of influenza was not noted in a single one of the patients. Therefore, in the direct observations which were of indubitable factual importance no proof was shown of any particular virulence of the virus. We must point out that not a single one of the authors analyzing this problem in retrospect directed proper attention to the results of artificial infection experiments in people presented above, which were performed during the pandemic.

The data of a number of authors speak against the existence of several pandemic viruses of different antigenic structure. Thus, Scoccia (1919) in Italy observed persons who had influenza in May-June 1918 and showed that they had not become sick in September. In India, Malone and McKendrick (1919-1920) made similar observations. Similar data have been reported by Hamilton and Leonard (1919), Speares (1919), and Waters (1920). However, there is also contradictory information on this subject. Included in it are the observations of Baker (1919), Winslow and Rogers (1920), Frost (1920), Jordan and Sharp and Vaughan (1921, 1922, 1923). It should be emphasized that all the authors listed, with the exception of Baker, came to the general conclusion that persons who had suffered from the pandemic infections had no immunity to the influenza which was observed again in 1920. However, what has been stated permits us with more reason to suspect the appearance of an antigenically altered variant after the pandemic rather than the existence of "several mutants" during it.

As a result of the pandemic infection, a tremendous

number of persons afflicted with influenza produced a mass group immunity; the only way in which this could have been overcome in the continuing epidemic process was the creation of a virus variant with hereditarily altered properties. This variability of the virus, as the current data suggest, occurred under the influence of immunity factors and was expressed in the epidemic outbreaks of 1920 and later.

After the 1918/19 pandemic the conglomeration of circumstances which might induce such an extensive distribution of epidemic influenza with a severe course and a high mortality rate did not recur for the next 35 years. The epidemic process assumed a relatively stable form with a general spread of the morbidity. However, during the period before 1933 no less than two active rises in the influenza morbidity rate were observed which were also of a pandemic nature, that is, they spread to a number of countries in different parts of the world for a comparatively short time (two years). This pertains to 1926-1927. According to Lukashevich (1941), after the 1918 pandemic there were definite influenza epidemics in Western Europe in 1927, 1929, and 1933; and in the USSR, in 1926 and 1934.

According to Gamaleya (1942), large-scale epidemic outbreaks of influenza were recorded in Spain, France, and Switzerland in December 1926; in January, in England and Germany. In Barcelona there were 160,000 cases of the disease in December. In the USSR the epidemic began at the end of January and reached a considerable intensity. In Moscow and Moskovskaya Oblast', according to Dobreytser, the morbidity rate figure per 10,000 persons varied from 58.6 to 246.4 depending on the age. The nature of the distribution of the morbidity rate with respect to time is of great interest, which can be judged by Fig. 180.

As seen for the two years from July 1925 through July 1927 there were two influenza outbreaks in Moscow separated by an interval of nine months.

It may be assumed that the epidemic which began in February 1926 was limited in its distribution from the time of the spring warm-up in April. However, this is not very probable, because in October, November, and December of the same year and January of 1927 the morbidity rate was low, and the epidemic outbreak, which lasted three months, began again only during the period from February through April 1927. The idea that even at that time there were two influenza virus varieties, different in their immunologic properties and created after the pandemic of 1918-1919, is more probable.

Despite the extensive distribution of influenza in 1926-1927, the course of the disease was, for the most part, benign and with a low mortality rate, which involved chiefly

old age and infancy. In the United States, according to Deutschmann's data (1950) the mortality rate from influenza at this time increased to 40 per 100,000 population, and in Canada, to 54.8.

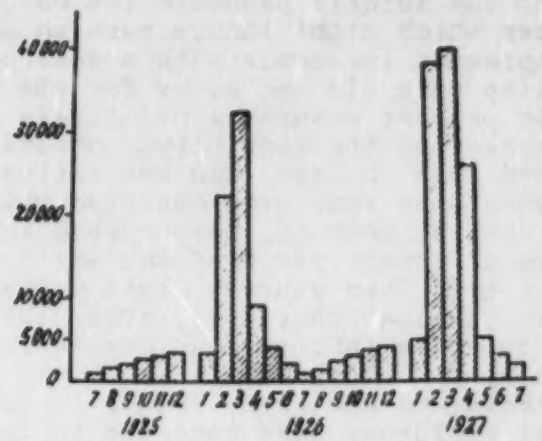

Fig. 180. Influenza Morbidity Rate in Moscow in 1925-1927 (Dobreytser, 1936).

Afterwards, according to the material of the same author, the highest influenza mortality rate was recorded in England and Germany in 1929, 1931 and 1933; in the United States and Canada, in 1929 and 1932. There is not only a correlation in the morbidity rates in Europe and America between the dates mentioned, but also there is a definite interval equal approximately to two years.

72. General Information on Influenza Epidemiology After 1933

After the isolation of the virus in 1933 and the creation of an experimental model of the infection in susceptible animals, opportunities were opened up in the study of the influenza problem which had not existed before. The most important basis had arisen for the development of current methods of investigation, diagnosis and differentiation of influenza from other similar diseases. As a result, epidemiology acquired a strong foundation for the discovery of many aspects of the epidemic process and, primarily, of

its etiologic nature.

According to Smorodintsev (1949), during the decade following the discovery of the influenza virus there were epidemic outbreaks of influenza in Europe in 1933, 1934, 1936, 1938, 1939, 1940, and 1943. The 1936 influenza outbreak in the United States was identified in retrospect as an outbreak of influenza B; the 1940 outbreak was accurately determined to be an outbreak of influenza B. The others were caused by A viruses. Table 91 from the work of Smorodintsev (1953) is presented below.

Table 91

Etiology of Influenza Epidemics After 1933

	1933	1934	1935	1936	1937	1938	1939	1940
USSR				A		A	a	B
England	A	a			A		Ba	
United States (East)	A	a		B	A		abB	b
United States (West)				B	A		b	BA
South America		A						Ab
Australia						B	A	a

	1941	1942	1943	1944	1945	1946	1947	1948	1949
USSR			A			A	B	A	Ab
England	a		baA			B			A
United States (East)	Ab		baA		bbBa			A	A
United States (West)			aA		bbB				
South America	b	a	A	b	bb				
Australia		A	aBa		bBa				

Key: A and B -- large-scale outbreak; a and b -- localized outbreak.

Against the background of a constant morbidity, which was particularly pronounced in the large cities, in 1936/37 a number of epidemics was observed which had the nature of a pandemic spread. At first, in December 1936, the epidemic of influenza A was noted in New York, and then it embraced all of North America, the European countries, China and Japan. In the USSR, the maximum number of cases

was observed at the end of February (in Moscow) and beginning of March (Leningrad); the duration of the outbreaks in both cities was 45 days (Dobreytser, 1937; Ansheles, 1945).

It is interesting to point out the fact that during this epidemic direct proof was obtained of the variability of the virus and the appearance of its antigenic varieties, which were formed during the course of the epidemic process. A comparison of 17 strains isolated from patients in England, Hungary and the United States in 1936-1937 by Magill and Francis (1938) showed that the majority of them differed from the influenza virus strains isolated previously, in 1933 and 1934. This apparently also explained the occurrence of an epidemic spread of unusual degree and extent; the epidemic did not, however, have a severe course and did not produce a high mortality rate.

Afterwards, the epidemic process assumed its previous, relatively stable form with an undulating and irregular distribution of the morbidity rate in time and in territory but with its obligate maximum during the cold part of the year.

This form of the epidemic process lasted until 1943, when influenza again assumed the nature of a pandemic, embracing a number of countries in a short time. According to Andrewes and Glover (1944), the existence of cases of influenza A in England was serologically recorded in April during a small and localized outbreak among service men. However, an epidemic broke out after the summer season, and in October and November it reached its maximum, and terminated in December. In the United States the first influenza outbreak was described by Adams, Thigpen and Rickard (1944) among children in the middle of November. In December, the epidemic spread throughout the country and, according to the data of the Commission on Acute Respiratory Diseases (1948), the morbidity rate among the service men increased by six times compared with October. Collins (1944) noted that the relative morbidity index for the country as a whole was 210 per 1000. Figs. 181 and 182, taken from the work of Sartwell and Long (1948), give us an idea of the size of this epidemic.

In Moscow, according to our data as well as according to the observations of Konova (1948), Gordina (1948), Edel'man, Kotina and Gavrilova (1948), the increase in morbidity rate began at the end of November and reached a maximum in the second ten-day period of December. The total duration of the outbreak, which was the one of greatest intensity after the 1936-1937 outbreak, amounted to 40 days. The approximate calculations show that this outbreak affected up to 18 percent of the city population (Shatrov, 1948).

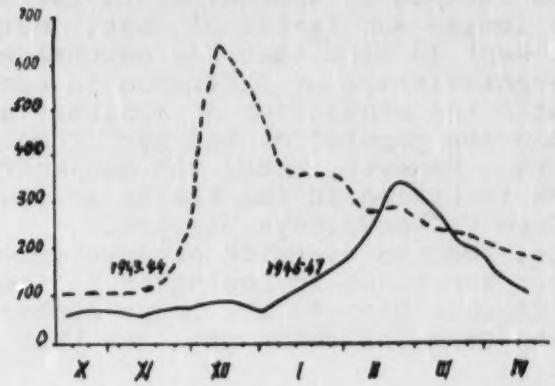

Fig. 181. Daily Sick List Per 1000 Persons in the United States Army During the Influenza A (1943-1944) and Influenza A-Prime (1946-1947) Epidemics (Sartwell and Long, 1948).

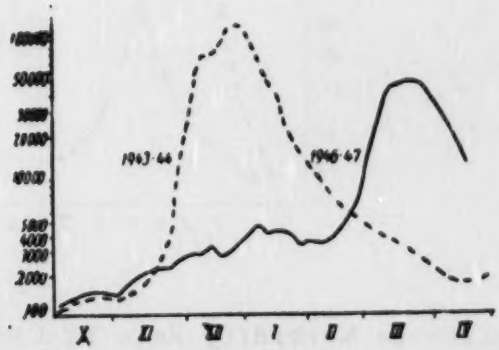

Fig. 182. Daily Record of Morbidity Rate Among the Civilian Population During the Same Epidemics (Sartwell and Long, 1948).

According to Ansheles (1945), the beginning of the outbreak in Leningrad pertains to 9-10 December, with the period of the greatest increase in the morbidity rate during

the first 10 days of January. Therefore, by comparison with Moscow the development of the influenza outbreak in Leningrad was delayed by approximately two weeks. In addition, it was longer and lasted 55 days. Noting these facts, it should be kept in mind that the unusual nature of the epidemic characteristics of influenza in Leningrad was associated with the conditions of military siege of the city, to which the population had been subjected for more than two years. However, until the beginning of the influenza outbreak in Leningrad two trains arrived daily from Moscow and from Volkhovstraya Station.

In Fig. 183 the dynamics of development of the 1943/ /1944 epidemic are shown in Leningrad in comparison with the 1936 epidemic. Despite the large number of cases, the mortality rate from influenza was less than in 1936 (Ansheles, 1945).

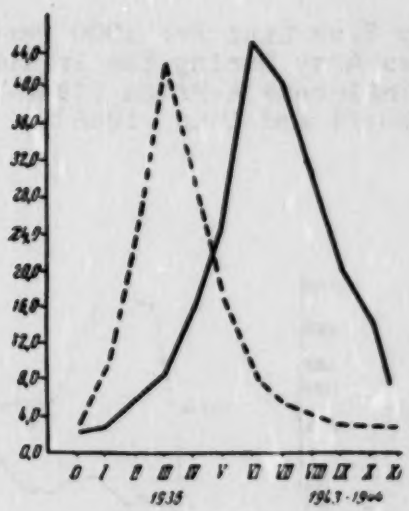

Fig. 183. Influenza Morbidity Rate in Leningrad in 1936 and in 1943/1944 (Ansheles, 1945).

According to Holland and Collins (1944), the mortality rate during the week of maximum morbidity rate in 1943 in the United States was 19.4 per 1000 population.

Strains isolated from patients in 1943 both in Moscow (Shubladze, 1944) and in foreign countries were similar to the PR8 virus but showed essential antigenic differences; an example is the Rhodes strain isolated by Salk, Menke and Francis (1944).

After 1944 no more widespread epidemics of influenza A were observed. The gradual evolution of the A virus, which occurred during the epidemic process, led to the formation of a new variant which was different in its antigenic structure -- the A-prime virus. As a result of this change a new pandemic spread of influenza occurred in America, Europe, the Asiatic countries and in the USSR from February 1947 through February 1952. The progress of the recent epidemic process, beginning with 1943, can be seen in a schematic form on Fig. 184, where the monthly distribution of the morbidity rate in Moscow is shown. The minimum morbidity rate of the preceding summer months is taken as the unit of comparison. The rise of morbidity rate in October 1947 corresponds to the pandemic wave of Asiatic influenza. For comparison, a diagram of influenza epidemics according to Stuart-Harris (1953) is given, based on the mortality rate statistics in 126 of the largest cities in the country.

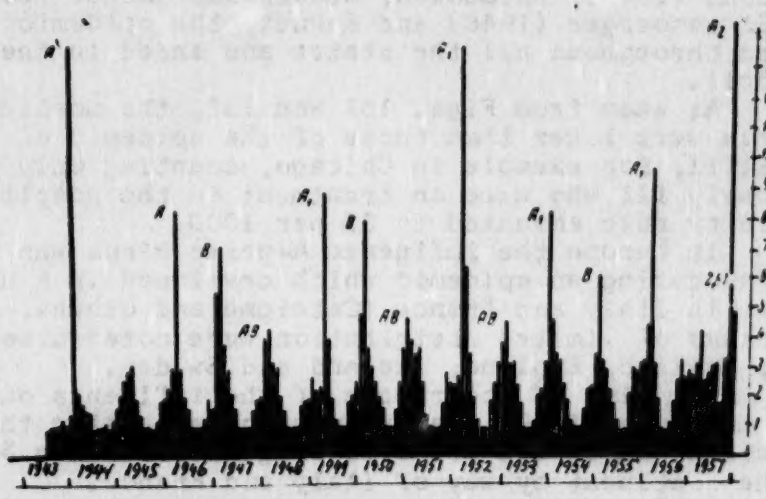

Fig. 184. Influenza Morbidity Rate in Moscow From 1943 Through 1957 by Months.

In Moscow and certain other cities of the USSR a small influenza A outbreak was noted after 1943-1944 as well as in the winter of 1945/1946; then, beginning with 1949, an epidemic disease occurred etiologically associated either with influenza B or with A-prime influenza. The participation of the A-prime virus in this epidemic process

was shown in the spring of 1949. The situation was different in foreign countries.

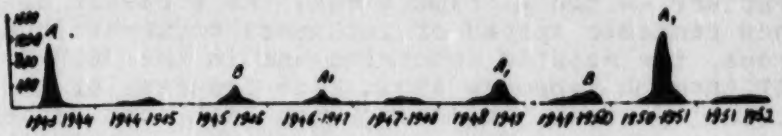

Fig. 185. Influenza Epidemic in England From 1943 Through 1952 (Stuart-Harris, 1943).

The first strain (CAM) with properties of an A-prime virus was isolated by Anderson and Burnet (1944) in Australia in May 1946; however, the influenza epidemic produced by this virus occurred in the United States during the middle of February 1947. According to the data of Sartwell and Long (1948), Rasmussen, Stokes and Smadel (1948), Loosli and Schoenberger (1948) and Burnet, the epidemic in March spread throughout all the states and ended in the first half of April.

As seen from Figs. 181 and 182, the morbidity rate figures were lower than those of the epidemic of 1943-1944, but still, for example in Chicago, counting only the very seriously ill who were on treatment in the hospitals, the morbidity rate amounted to 35 per 1000.

In Europe the influenza A-prime virus was found in 1948/49 during an epidemic which developed in a number of cities in Italy and France (Cateigne and others, 1949). Outbreaks of limited distribution were noted also in Austria, Holland, England, Iceland and Sweden.

The time of occurrence of the influenza outbreaks gave Andrewes (1949) the grounds for supposing that the infection had spread from its original focus in Sardinia to the continent by way of Italy and France.

In Fig. 187 Andrewes' map of the suggested path of the epidemic is shown.

In Sardinia the epidemic appeared in the North of the island and involved both the urban population and the shepherds in the remote mountain areas. Stuart-Harris (1953) says in reference to this that "There is no clue with which to explain the causes of occurrence of the outbreak in Sardinia." However, we believe that in this case the "clue" is the fact of the existence of ports, naval and air-force bases on the island associated with a turnover of Americans, who may have served as sources of importation of

the infection and for the occurrence of the epidemic among the population.

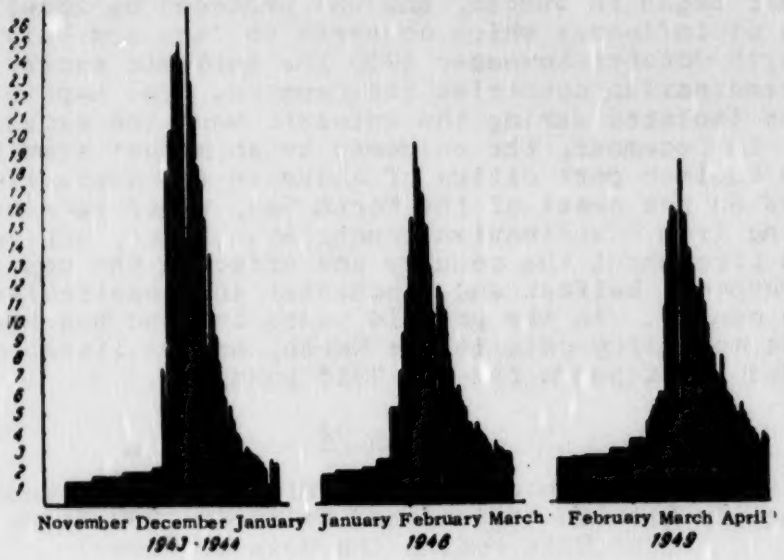

Fig. 186. Influenza A Epidemic (1943/44 and in 1946) and A-prime (1949) in Moscow.

Fig. 187. Spread of A-prime Influenza in Europe in 1948-1949 (Andrewes, 1949).

The epidemic of influenza A-prime in 1950/51 spread quite widely and involved Europe, Africa, and North America, Japan and a number of other Pacific islands. In Europe the epidemic began in Sweden, and was preceded by local outbreaks of influenza which occurred in June and July. Beginning with October-November 1950 the epidemic spread to all the Scandinavian countries and Denmark. The A-prime virus strains isolated during the epidemic were the same.

In December, the epidemic began almost simultaneously in the English port cities of Aberdeen and Newcastle, located on the coast of the North Sea, which receive ships arriving from Scandinavian countries. Later, influenza spread throughout the country and affected the population of Liverpool, Belfast and Manchester in a particularly severe manner. In the past 14 years England has had its highest mortality rate in the North, and in Liverpool it exceeded the figures for the 1918 pandemic.

Table 92

Mortality From Pneumonia and Influenza and Pneumonia Morbidity Rate in England and Wales in 1950/51 by Weeks (The Date Ending the Week is Given)

Date	No who died of pneumonia	No. who died of influenza	No. of cases of pneumonia
9/XII 1950 r.	236	34	573
16/XII	289	33	684
23/XII	351	54	847
30/XII	383	102	962
6/I 1951 r.	770	458	1 774
13/I	982	890	2 538
20/I	843	1 099	3 047
27/I	790	1 239	5 572
3/II	830	1 269	3 513
10/II	662	864	3 091
17/II			
24/II			
3/III	396	178	404
10/III	297	88	233

In Table 92 the mortality rate figures are presented for influenza and pneumonia and the pneumonia morbidity rate is given for England and Wales during this epidemic.

The mortality rate in England during this epidemic is shown graphically in Fig. 188, where the data of successive winter seasons are compared.

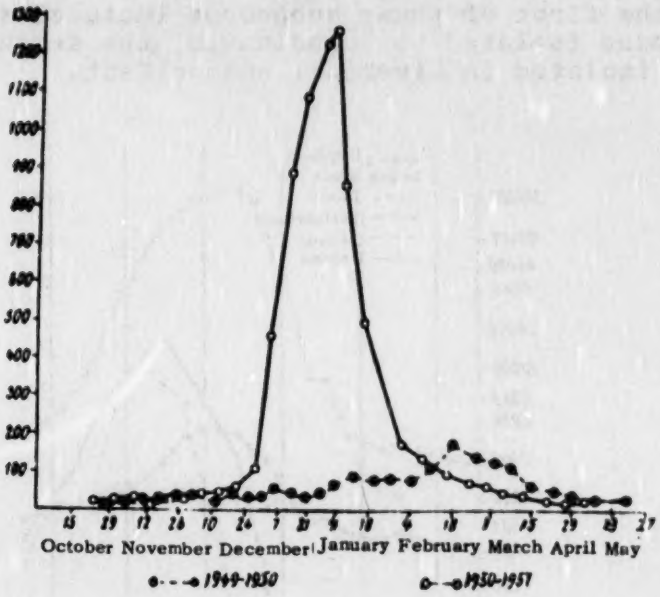

Fig. 188. The Mortality Rate From Influenza in the Cities of England in 1949/50 and 1950/51 (Isaacs and Andrewes, 1951).

Of the other countries a high mortality rate was noted in Spain.

In Czechoslovakia this epidemic caused about 400,000-500,000 cases, after involving a considerable part of the population of the country. These official statistical data, in the opinion of Blaskovic and co-authors (1952), should be increased by at least two to four times. The morbidity began to increase vigorously in December 1950 and reached a maximum in January 1951. The course was benign, and the mortality rate was low.

In Holland the wave of influenza A-prime struck from December 1950 through February 1951, involving nine percent of the population, after which it declined. However, in April a slight increase in influenza B was observed (Verlinde, 1952).

In Fig. 189 a diagram is given of the morbidity rate figures for 1950/51, in accordance with which the movement of influenza may be followed in six countries. As in 1949, the strains isolated belong to the A-prime virus type, but they still could be subdivided into two subgroups.

According to the investigations of Isaacs and Andrewes (1951), the first of these subgroups included strains similar to those isolated in Scandinavia; the second, similar to those isolated in Liverpool and Belfast.

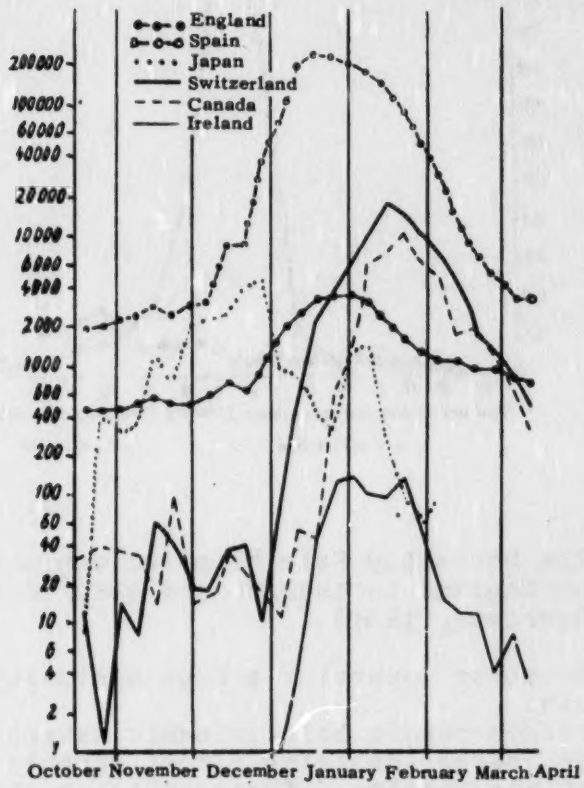

Fig. 189. Influenza Morbidity Rate Figures in 1950/51 in Six Countries (Andrewes, 1954).

Incidentally, these results served as the grounds for the hypothesis that one group of epidemics occurred in Scandinavia from previous summer outbreaks, while the other was supposedly associated with the importation of the virulent Liverpool virus from the "Southern Hemisphere or from the East." It should be noted, however, that this hypothesis is purely arbitrary and is not supported by facts.

In Japan, according to the data of Fukumi, Yamamoto and Nichikawa, Kitayama, Sinawaka and Nakano (1951), in the

winter of 1950/51 the epidemic of A-prime influenza grew, beginning with the end of October, and extended from the prefectures in the West to the East or Northeast. After a year, during the first ten days in March 1952, an epidemic of A-prime influenza, which embraced the entire country, was repeated in Japan. According to the investigations of Fukumi, Nichikawa and Kitayama (1953) the A-prime virus strains isolated during both epidemics were the same; a comparison of them with other virus strains obtained from France, Sweden and the United States, made it possible for the authors to conclude that the epidemics in Japan represented a part of the pandemic spread of A-prime influenza.

The investigations mentioned above indicate a single important fact, namely, that the A-prime virus had been found earlier in 1949/50 during localized outbreaks of influenza observed in Japan, the majority of which, however, were related to the B virus. Isolated strains (TM and Masuyama) belonged to the Scandinavian type of A-prime virus. We find this communication interesting in view of the fact that in Japan, as in the other countries, the spread of A-prime influenza was of the nature of epidemics of gradually increasing strength; several years were required for them to develop into a pandemic.

According to the data of the World Health Organization (WHO, 1953), A-prime influenza in 1951-1953 again assumed a pandemic spread in the Northern Hemisphere, including the Pacific islands (Guam, Marshalls and Philippine Islands) in the winter of 1952/53; Japan, in February 1953; the United States at the end of 1952, and European countries at the beginning of 1953, in which an increase in the morbidity rate was observed everywhere and simultaneously.

According to Davis and Dauer (1953), in the summer and autumn of 1952 there were only small localized outbreaks of influenza in the United States. In December, a rise in the morbidity rate was observed, which in February led to the epidemic spread of influenza in a number of states. Thus, while the mortality rate from influenza in February 1952 did not exceed 8.4, in February 1953 it reached 26.4 per 100,000 population.

According to Herzberg (1953) the epidemic of A-prime influenza in 1952/53 developed in the following manner. In the middle of December 1952 outbreaks of influenza occurred in Japan and in the United States. At the beginning of January 1953 an increase in the number of cases occurred in France. From the middle to the end of January outbreaks of influenza developed in Holland, Belgium, Germany, England, Finland and Iceland; at the end of January, in Switzerland, Austria, Central America, Jamaica, Canada, and Alaska. At the beginning of February the influenza epidemic included

Portugal, Italy, Greece, Yugoslavia, Egypt, Morocco and the Pacific islands.

In March and April the rise in morbidity rate was noted in the majority of other countries as well as in Corsica and Costa Rica; in April, in Israel; in May, in Uruguay. At that time Australia was free of influenza. It is of some interest to note that the winter of 1952/53 in the USSR was not associated with the development of an influenza epidemic. There was only a moderate seasonal rise in mobridity rate associated with the A-prime and B viruses.

In Finland, this epidemic, which reached its maximum development in the autumn of 1952, produced 384,000 cases, which constituted about 10 percent of the population (Penttinen, 1953).

Francis (1953) summarizes the data on the etiology of influenza epidemics in the United States in the following manner (Table 93).

Table 93

Etiology of Influenza Epidemics in the United States

Type of virus				
A of swine	A	A_1	B	C
1930/31	1932/33			
	1934/35			
	1936/37		1936 (beginning of the year)	
	1938/39			
	1940/41		1940 (» »)	
	1943/44		1945 (» »)	
		1947 (beginning of the year)		+
		1950 (» »)		+
		1951 (» »)		
			1952 (» »)	±
		1953 (» »)		

In Fig. 190 data are presented on influenza epidemics in the United States on the basis of a comparison of investigations and statistics of the influenza and pneumonia mortality rate according to Collins and Lehmann (1953), which give us an idea of the mortality rate but not of the morbidity rate, since the mortality rate from influenza has

been different during its various epidemics.

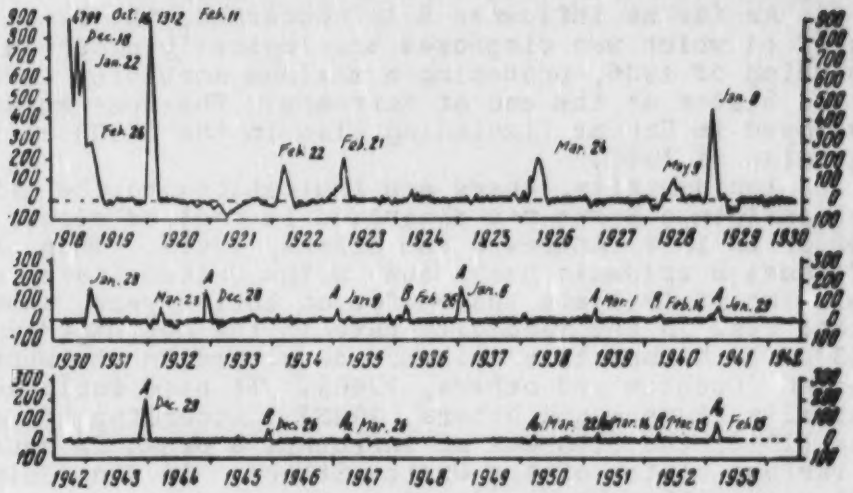

Fig. 190. Mortality Rate From Influenza and Pneumonia in the United States From 1918 Through 1953 (Collins and Lehmann, 1953).

The figures designate the degree to which the average yearly figures are exceeded.

In describing the history of A-prime influenza, Francis (1953) points out that the virus of A-prime influenza circulated in Australia beginning with 1946; in the United States in 1947 it produced a large-scale epidemic, and in the winter of 1947/48 it included various parts of Western Europe. In 1948, it produced a widespread epidemic in Europe, and in 1950/51 was observed throughout the world.

In evaluating the data presented it may be seen that from the time of discovery of the influenza A virus three pandemics were observed associated with the virus pathogen: in 1936/37, in 1943/44, and in 1947-1952. Between these pandemics a relatively stable epidemic process was established characterized by an alternation of yearly seasonal variations in the influenza morbidity rate and outbreaks of varying degrees of intensity. Each of the three pandemics named was associated with a virus which showed essential changes in its antigenic structure. This applies particularly to the last pandemic, associated with the new A-prime variant formed on the basis of the A virus which preceded it. These data also show that outbreaks in various countries

preceded the development of the influenza pandemic produced by the A-prime virus, while the actual spread of this virus throughout the world required several years.

As far as influenza B is concerned, the first epidemic of it which was diagnosed etiologically occurred at the beginning of 1936, producing a maximum morbidity in the United States at the end of February. The next epidemic developed in Europe (including also in the USSR) at the beginning of 1940.

Incidentally, there are indications to the effect that influenza B was not diagnosed in England either in 1940 or in 1941 (Andrewes and others, 1941). Then, the new influenza B epidemic broke out in the United States at the beginning of 1945 and lasted for an entire year, showing a second rise in the morbidity rate in the autumn (Francis, 1953). In Europe this epidemic developed in the winter of 1945/46 (Dudgeon and others, 1946). It also included Australia (Burnet and others, 1946). According to Jackson's data (1946) the outbreak of influenza B began in June 1945 in various states of the United States. In June-July of that year outbreaks developed in the West Indies, in the Hawaiian Islands, Guam, Philippine Islands, Panama, and Alaska. In July and August the disease had spread to South America. In England the outbreak developed from December 1945 through March 1946; in the USSR, later, from December 1946 through March 1947. It is interesting to note that during this outbreak the new serological variety, the B virus (Bon or Kri type strains) was isolated for the first time.

The last great influenza B epidemic occurred in 1949/50 in the USSR; in 1952, in the United States. In England the influenza B epidemic included many cities in the winter of 1951/52 (Anderson and others, 1953).

In the USSR the influenza outbreak in 1949/50 included the majority of the cities of the country. In Moscow, the maximum number of cases was found in December-January, and in certain cities even earlier. For example, in Krasnodar the maximum morbidity rate was observed in November-December 1949 (Neklyudova, 1951).

In Figs. 186 and 191 data are presented on the dynamics of development of the last influenza epidemics in Moscow. The longer and less intense nature of the development of the influenza B outbreaks in contrast to the stormy but brief outbreaks of A and A-prime influenza attracts attention. Although Francis (1953) also writes about a pandemic spread of influenza B in 1952 this assertion seems unfounded to us. There is more basis for speaking about a pandemic spread of influenza during the period from 1945 through 1947.

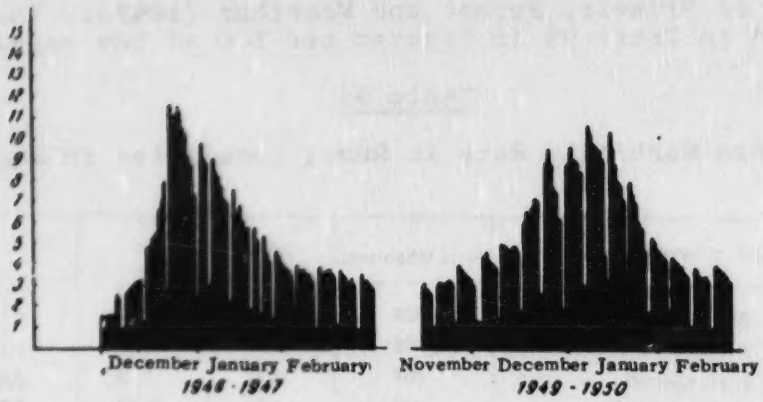

Fig. 191. Epidemic of Influenza B in 1946/47 and in 1949/50 in Moscow.

73. Morbidity Rate and Mortality Rate

As will be shown below, the epidemic process in influenza is expressed in three main forms, which attests to the inequality of distribution of the morbidity rate in time and by territories. The latter is reflected in the fact that with modern means of transportation, which provide a lively circulation of people between inhabited places, the morbidity rate in the same country is different in different cities.

Thus, for example, in the spring of 1949 an outbreak of A-prime influenza in Moscow produced an increase in the morbidity rate by eight times compared with the minimum morbidity rate recorded in July-August; in Khar'kov, by three times; and in Kiev and Krasnodar it was not so pronounced. A similar phenomenon could be observed not only in various localities but even in various places of the same city. In the same 1949 epidemic the morbidity rate figures fluctuated within limits of from 47 (Moscow Electric Plant) to 192 per 1000 (Moscow Factory imeni The Eighth of March). During the influenza A-prime outbreak in 1953/54 in Pervomayskiy Rayon of Moscow the morbidity rate figures at various enterprises ranged within limits of from 64 to 416 per 1000 (according to the data of Topolyanskaya, Lev, Lurina, Berenshteyn, Gerasimova, 1954).

Interesting observations on the morbidity rate in the rural localities of the same region have been given in

the work of Pickels, Burnet and McArthur (1947). They are presented in Table 94 in figures per 100 of the population.

Table 94

Influenza Morbidity Rate in Rural Localities in England

Name of inhabited place	No of inhabitants	1933	1937	1943
Aysgarth	438	1,37	17,81	4,11
Thoralby	298	6,38	13,76	6,71
West-Bretton	367	14,17	5,72	3,81
Kerperby (?)	272	1,84	21,32	7,72
Redmire	350	1,14	20,28	12,29
Askrigg	628	2,87	11,31	9,24
Bainbridge	560	5,4	3,6	7,2
West Witton	377	4,24	9,28	3,45

Three years are compared, because at that time large-scale epidemics were observed in England which were connected with the same type A virus. The authors believe that on the basis of the data presented it is possible to evaluate the duration of the post-infectious collective immunity in small hamlets, which, to a considerable degree, remained for four years. They see the proof of this in the correlation of the low morbidity rate figures in 1933 with the high figures in 1937 (Aysgarth, Kerperby [?], Redmire, Askrigg) and in the reverse relationship (West-Bretton). Subsequently, in 1943, that is after six years, no such correlation was observed.

In our opinion, another fact deserves attention, namely, that in the same area different inhabited places showed different morbidity rate figures during three epidemics. In 1933 they ranged from 1.14 to 14.17; in 1937, from 3.6 to 21.32; in 1943, from 3.45 to 12.29. It should be noted that in England the most intense epidemic occurred in 1936/37.

Above, we have mentioned the high mortality rate which occurred in Liverpool during the influenza epidemic in England in the winter of 1950/51. Let us keep in mind the fact that Liverpool is a very large port and industrial center, which suffered severely from air bombardments of the Hitlerite air force. The result of this was an acute shortage of living space and overcrowding of the city's population. According to Logan (1951), the epidemic in Liverpool lasted 21 days, and "...even if we do not consider

the victims of the air bombardments, the number of deaths which occurred in this time may be compared only with the cholera pandemic of 1849, after which the city's vital statistics have not shown so much loss of life." The worst week in 1918/19 with respect to the morbidity rate was marked by 638 deaths, while from 6 through 13 January 1951 894 persons died; of these, 453 directly from influenza and pneumonia; 241 from heart and circulatory diseases. The highest mortality rate was noted among patients over 50 years of age; the main causes of the fatal outcomes were pneumonia and toxic myocarditis. In young persons, not uncommonly cyanosis was observed similar to what occurred during the 1918/19 pandemic.

At the same time, the weekly mortality rate in Manchester amounted to 102.2 per million; in Birmingham, 67.3; in London, 41.6. In a number of cities in England influenza had a much milder course, even though it caused a high morbidity rate. An example of this is Salford, a city located a total of only 30 miles from Liverpool. In it 100,000 out of the 180,000 population became sick with influenza with 49 fatal cases of the disease, which constitutes a mortality rate of 0.049 percent.

Logan points out that in England as a whole from January through February 1951 about 50,000 persons died in direct or indirect association with the influenza epidemic.

The influenza mortality rate statistics are the basic material, on the basis of which the influenza morbidity rate may be judged in foreign countries, because no regular records of the disease are kept, and it is recorded only from case to case by way of scientific interest. In addition to the figures already presented we are presenting information on the annual influenza mortality rate for a number of countries presented in the work of Deutchmann (1954), a worker in the statistical bureau of the World Health Organization of the United Nations (Table 95).

The development of influenza epidemics, the morbidity rate and mortality rate in a considerable measure are determined by the character of contact between people, the living and working conditions, and the condition of the immunity in the population. An important factor requiring special attention and consideration is the age composition of the patients.

Data existing in the literature constitute evidence of the different degrees of susceptibility of various age groups of the population to influenza. In 1833, Filatov pointed out that influenza most often involves children and chiefly those from six months to five years of age. Koltypin (1938, 1935), in a study of the clinical characteristics of influenza in children, noted their very high degree of

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susceptibility from six months to three-five years of age.

Table 95

Influenza Mortality Rate in Various Countries in 1920-1951

Country	Year										
	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930
Africa:											
Union of South Africa		82,8	50,6	25,3	30,7	24,4	39,0	24,4	26,9	19,4	23,8
America:											
Canada		14,7	36,9	54,6	19,7	32,4	54,8	35,9	47,9	71,6	24,2
United States	70,9	11,4	31,2	44,3	19,4	29,7	40,8	22,6	45,3	55,5	19,4
Chile	168,0	191,0	125,0	449,0	138,0	204,0	171,0	121,0	138,0	267,0	214,8
Uruguay	2,0	12,2	3,3	14,1	11,5	7,2	7,3	12,8	3,2	9,3	—
Asia:											
Japan	194,0	18,2	22,1	11,2	11,0	18,1	7,6	13,2	15,5	13,4	8,1
Australia	8,4	12,0	6,4	21,3	10,0	5,9	12,5	6,9	12,7	15,9	4,3
New Zealand	40,2	8,6	5,3	31,6	6,9	6,5	21,3	9,5	17,4	21,1	9,2
Europe:											
Belgium	39,2	30,4	46,4	29,0	27,6	26,5	34,5	32,1	25,0	47,4	15,3
Czechoslovakia	52,4	8,8	16,5	5,3	4,2	8,4	10,5	30,4	7,3	19,1	4,6
Denmark	97,0	4,9	46,1	21,4	18,8	12,1	13,8	55,8	15,0	39,5	9,5
Finland								19,6	7,2	39,7	5,2

Table 95 [continued]

Country	Year										
	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930
France	28,7	17,6	—	—	—	14,1	17,1	38,1	15,3	40,2	8,3
Germany	96,0	27,2	64,2	38,8	23,5	22,4	25,8	46,3	19,4	57,5	12,0
Greece	—	72,7	72,9	55,3	76,0	55,7	41,4	87,1	52,8	11,2	52,8
Hungary	71,6	6,0	16,9	5,4	12,4	7,6	5,4	20,0	5,0	15,3	4,1
Iceland	55,5	83,2	25,0	20,6	34,7	7,1	22,8	6,8	16,3	19,9	4,7
Ireland	22,4	20,9	60,0	20,6	60,0	39,2	22,0	71,0	29,1	56,3	21,0
Italy	67,2	11,3	36,1	23,9	22,5	22,5	34,4	22,5	25,3	48,2	17,2
The Netherlands	35,8	6,7	52,5	11,0	8,1	8,7	18,8	42,6	18,8	57,1	8,5
Norway	13,8	2,1	20,0	12,6	3,6	2,0	3,5	19,7	5,5	17,2	1,7
Spain	83,7	27,2	36,9	38,3	31,9	28,3	22,5	39,1	14,9	23,4	10,3
Switzerland	90,7	11,8	44,0	13,9	38,2	28,5	20,3	67,1	19,1	44,2	12,7
United Kingdom:											
England and Wales	28,2	23,7	56,3	22,0	49,0	32,7	22,9	56,7	19,6	73,4	12,6
Northern Ireland	44,5	25,1	70,1	33,0	81,6	55,8	38,4	59,6	31,0	107	20,6
Scotland	6,0	27,2	75,7	10,9	51,2	25,6	23,2	41,7	19,8	71,1	14,4

Country	Year										
	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941
Africa:											
Union of South Africa	17,4	45,6	17,3	15,2	60,7	17,8	25,1	15,7	13,4	16,3	12,4
America:											
Canada	31,0	40,4	37,8	18,7	31,3	28,5	47,7	21,2	35,2	24,5	21,0
United States	26,5	30,6	26,4	17,3	22,2	26,4	29,5	12,7	16,4	15,3	15,8
Chile	120,4	134,0	245,5	173,6	185,6	161,8	127,3	156,6	109,2	108,1	85,3
Uruguay	—	—	—	—	12,5	3,6	7,1	8,5	3,4	4,7	2,5
Asia:											
Japan	24,0	8,1	7,1	14,8	4,4	12,4	4,2	10,7	8,2	4,4	5,9
Australia	8,7	5,3	13,3	14,1	17,4	7,0	5,8	9,0	12,7	5,0	4,9
New Zealand	15,3	4,6	7,0	12,6	7,4	9,4	7,3	8,8	11,0	7,7	4,9
Europe:											
Belgium	31,2	38,0	38,9	19,6	31,7	23,0	24,6	21,3	36,9	24,5	25,0
Czechoslovakia	12,0	7,9	15,9	4,3	13,9	6,5	11,6	6,1	11,9	6,2	14,2
Denmark	34,5	32,7	23,9	6,3	13,6	19,5	20,4	9,8	10,8	15,5	13,9
Finland	8,8	8,7	20,7	5,2	5,8	47,7	15,0	27,0	16,2	24,8	14,3
France	21,8	11,1	21,8	6,3	18,3	10,7	--	—	—	20,7	5,7

Table 95 [continued]

Country	Year										
	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941
Germany	30,0	15,9	41,3	14,2	32,9	28,9	26,0	14,0	28,0	—	—
Greece	87,5	76,3	131,7	43,1	77,2	43,0	100,5	47,9	—	—	—
Hungary	7,7	10,8	9,2	5,6	10,6	5,4	6,3	7,5	—	—	—
Iceland	20,2	0,9	12,4	5,3	19,9	4,3	74,2	—	22,6	1,7	31,2
Ireland	50,2	56,1	60,5	23,2	32,4	23,0	94,0	23,9	37,2	28,0	44,6
Italy	32,2	32,1	28,7	19,6	27,5	24,8	28,0	19,6	21,7	15,7	16,3
The Netherlands	39,9	17,4	21,2	10,7	18,9	18,4	36,7	12,7	21,8	17,8	47,9
Norway	9,1	6,4	7,3	1,7	6,4	4,4	5,7	1,8	13,6	4,2	13,8
Spain	31,2	20,6	32,6	22,7	32,9	16,5	18,1	20,0	19,6	16,9	24,4
Switzerland	45,5	46,9	27,6	11,2	45,6	15,2	16,4	28,6	47,5	37,7	7,9
United Kingdom:											
England and Wales	33,0	30,0	52,0	12,7	16,7	14,1	41,8	10,8	19,4	28,6	17,7
Northern Ireland	46,3	36,4	69,3	26,8	32,7	21,4	89,7	20,1	31,0	51,3	34,4
Scotland	26,2	35,3	41,3	12,2	29,5	13,3	54,1	7,9	18,3	37,2	14,6

Country	Year									
	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951
Africa:										
Union of South Africa	8,8	11,1	7,1	3,5	2,4	3,5	4,3	3,8	6,6	—
America:										
Canada	10,5	20,5	15,6	9,0	13,0	8,8	7,1	8,2	6,9	18,4
United States	8,1	12,9	13,1	7,7	6,3	5,3	3,5	3,1	4,4	—
Chile	63,4	49,7	47,0	43,5	32,8	23,0	24,8	25,6	16,1	—
Uruguay	2,6	5,6	—	13,6	6,6	11,2	8,5	7,0	4,2	—
Asia:										
Japan	5,4	4,6	—	—	—	2,4	0,6	0,6	1,5	—
Australia	7,7	6,1	2,5	2,2	2,8	2,0	4,9	1,5	4,3	—
New Zealand	16,1	4,2	4,0	3,3	6,7	1,9	3,0	2,6	4,3	—
Europe:										
Belgium	19,4	18,7	20,2	22,8	21,5	18,5	12,2	14,6	9,8	17,1
Czechoslovakia	3,7	—	—	—	—	—	—	—	—	—
Denmark	2,9	2,5	5,8	1,7	4,1	3,3	0,7	3,8	6,1	3,4
Finland	9,1	4,9	6,8	2,8	1,4	9,9	2,6	14,0	1,8	8,8

Table 95 [continued]

Country	Year									
	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951
France	4,8	3,9	11,8	5,6	5,9	4,5	3,8	29,3	4,5	19,6
Germany	—	—	—	—	4,5	6,4	7,1	18,2	7,0	10,8
Greece	—	—	—	—	—	—	—	—	—	—
Hungary	—	—	—	—	—	—	—	—	—	—
Iceland	1,6	28,8	3,2	—	5,3	7,4	0,7	7,2	3,5	—
Ireland	13,1	15,7	27,6	10,4	26,0	21,9	6,7	9,4	10,9	77,7
Italy	9,4	9,1	8,7	6,2	7,9	6,9	11,4	12,5	3,7	9,3
The Netherlands	10,2	9,4	15,7	5,6	9,4	5,7	3,5	25,4	6,2	15,7
Norway	2,4	3,0	2,7	1,0	2,5	1,8	1,3	2,0	3,4	2,4
Spain	17,7	15,2	19,2	11,6	7,6	13,4	10,0	8,7	6,4	—
Switzerland	7,1	4,6	28,4	13,6	13,0	15,5	5,6	13,2	5,5	24,9
United Kingdom:										
England and Wales	8,8	33,3	10,3	7,0	13,0	7,9	2,9	13,0	8,9	36,1
Northern Ireland	15,0	26,0	16,2	10,5	28,6	20,4	5,6	7,1	11,5	64,4
Scotland	6,5	24,8	7,8	4,4	13,9	6,7	2,7	7,7	7,5	21,8

According to Dobreytser (1936), the maximum morbidity and mortality rates from influenza during the pandemics of 1918/19 and 1926/27 also occurred among children (Table 96).

Table 96

Mortality Rate According to Age and Sex in Moscow (Per 100,000 Persons of the Corresponding Group)

Age in years	Year					
				1926		
	1917	1918	1919	male	female	both sexes
0—4	7,9	9,8	22,1	18,2	13,6	15,9
5—9	0,2	0,6	2,6	0	0	0
10—14	0	0,5	0,1	0	0	0
15—19	0,1	0,7	1,8	0,2	0	0,1
20—29	0,1	1,4	2,3	0,2	0,2	0,2
30—39	0,1	2,1	3,7	0,1	0,3	0,2
40—49	0,4	2,1	4,9	0,8	0,3	0,5
50—59	0,6	3,4	9,7	1,3	1,2	1,2
60 and over	4,8	10,9	23,9	2,2	5,9	5,1

The graphic indices of the morbidity and mortality rates in Moscow in 1926 are shown in Fig. 192.

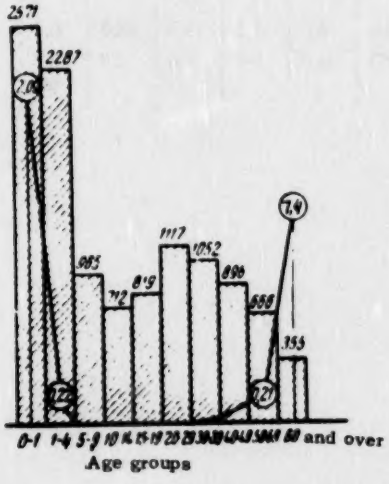

Fig. 192. Number of Cases of Influenza in Moscow in 1926 per 10,000 Persons of the Corresponding Age and the Mortality Rate Among the Patients (Dobreytser, 1936).

According to Frost (1920), Frost and Sydenstricker (1919), the maximum morbidity and mortality rates from influenza in 1918 in the United States occurred between the ages of five and ten (Figs. 193 and 194).

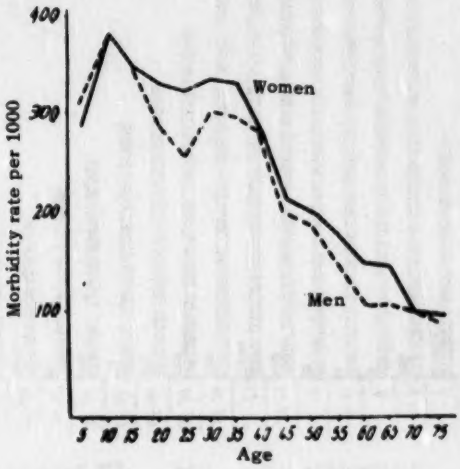

Fig. 193. Influenza Morbidity Rate in the United States by Sex and Age in 1918 (Frost, 1920).

In Switzerland the number of cases of influenza in 1918 amounted to 2,000,000 persons (half of the entire population). In this year 21,000 persons died from influenza, which constituted a mortality rate of about one percent. The maximum mortality rate in Switzerland occurred between ages 20 and 29.

In 1936 in the United States, according to Francis (1937), during the epidemic children of school age were most affected. Therefore, many schools were closed on account of the absence of students.

Collins (1944) indicates that in 1943/44 the maximum morbidity rate was noted among the children. It amounted to 300 for ages zero to five, and 325 per 1000 between the ages of five and nine.

In Fig. 195 data are presented from his work which show that the reduction in the morbidity rate in these years occurred chiefly because of the reduction in the morbidity rate in the older age groups.

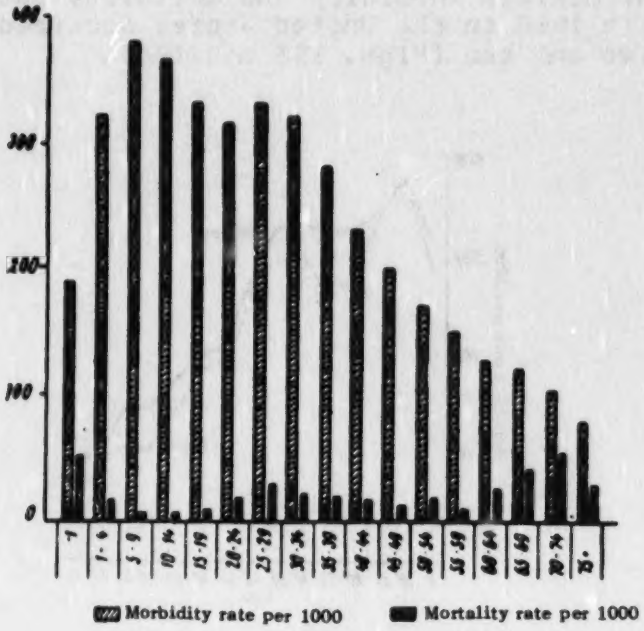

Fig. 194. Morbidity and Mortality Rates From Influenza in the United States in Different Age Groups in 1918 (Frost and Sydenstricker, 1919).

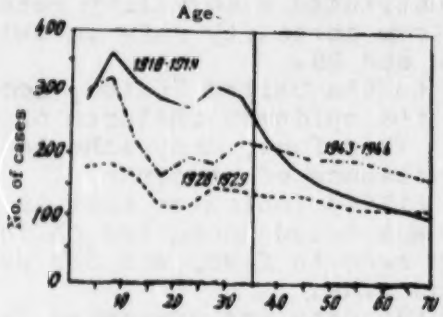

Fig. 195. Morbidity Rate in Different Age Groups During the Three Influenza Epidemics in the United States (Collins, 1944).

The epidemic of 1943/44 in the USSR was studied in detail by Shatrov (1948). According to his data, at various institutions and in Moscow as a whole the children constituted a large proportion of the patients. This can be judged by the data of Table 97.

Table 97

Morbidity Rate According to Ages in Percentages of the Population of a Given Age

No of institution	Age				
	0 4 years	5 8 years	9 14 years	adults	on the average
1	38,3	22,2	35	18,8	21,6
1/2	11,5	14	23,3	14,6	15,3
3	18,2	16,1	22,5	17	17,5
4	22,9	20,4	43,6	27,9	28,5

On the average, this amounted respectively to the following: 24.5, 19.1; 34.6; 21.8; 22.9, and in Moscow, 11.2, 12.3, 8.9, and 9.3.

It is of some interest here to give Lancaster's comparative data on the influenza mortality rate in Australia among different age groups from 1908 through 1945 (Table 98).

As the result of an analysis of the statistical material, Logan (1951) believes that in the past 30 years a definite shift has occurred in the influenza mortality rate in the direction of older ages. He demonstrates this graphically with a diagram (Fig. 196).

According to our observations, the age distribution of the morbidity rate in recent years permits us to conclude that the maximum involvement is of the children's groups. This may be judged by Figs. 197-199, in which a comparison is presented of the relative influenza figures for 1946, 1947, 1948 and 1949.

Table 98

Mortality Rate per Million of the Population per Year in Australia in Different Age Groups

Years	Sex	Age in years									Total
		0–4	5–14	15–24	25–34	35–44	45–54	55–64	65–74	75 and over	
1908—1910	Male	116	16	15	37	58	117	215	641	1 754	99
1911—1920	»	165	43	177	558	546	489	443	711	1 772	347
1919	»	880	305	1 549	5 046	4 746	3 987	2 627	2 811	3 454	2 617
1921—1930	»	100	18	35	42	83	145	260	474	1 544	114
1931—1940	»	140	21	31	36	66	119	211	408	1 283	109
1941—1945	»	121	9	8	7	19	38	74	170	548	49
1908—1910	Female	83	14	19	25	59	72	220	677	2 225	93
1911—1920	»	152	47	154	374	322	319	456	810	1 972	266
1919	»	816	331	1 338	3 208	2 678	2 363	2 743	1 977	3 211	1 825
1921—1930	»	92	18	30	58	88	103	180	421	1 673	103
1931—1940	»	128	17	24	39	63	81	134	346	1 242	96
1941—1945	»	118	9	3	9	18	23	45	136	534	44

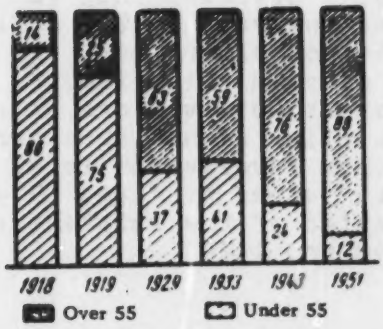

Fig. 196. Distribution of Influenza Mortality Rate During Various Influenza Epidemics in England. The Percentile Relationship of Fatal Outcomes in Persons Older and Younger than 55 Years of Age (Logan, 1951).

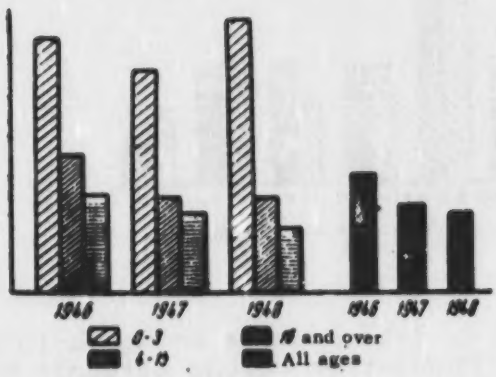

Fig. 197. Morbidity Rate Among Children and Adults in 1946-1948 in Moscow.

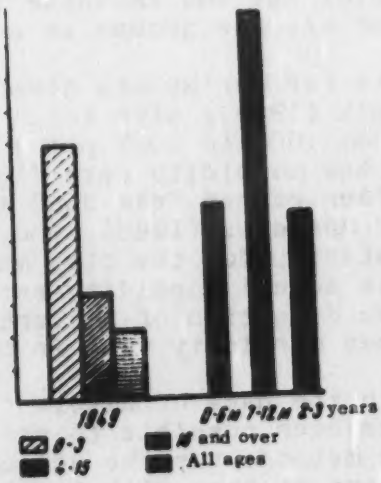

Fig. 198. Morbidity Rate Among Children and Adults in 1949 in Moscow.

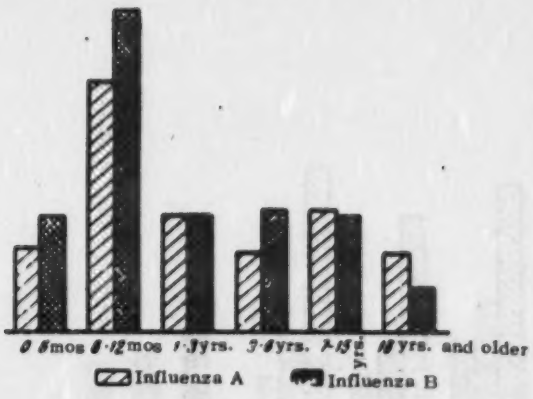

Fig. 199. Morbidity Rate Figures Among Various Age Groups of the Population During Epidemic Outbreaks of A and B Influenza.

Bychkova (1951), studying the etiology of influenza-like diseases in the newborn in lying-in homes, concluded that during two influenza epidemics there was not a single case of influenza which could be proved by laboratory methods of diagnosis. The highest morbidity rate figures for the 1949 epidemic in Moscow were in children from six months to one year of age and amounted to 1573.65 per 10,000, whereas for all age groups as a whole it equaled 707.4 per 10,000.

Similar data for Gor'ky are given in their work by Grennaus and Aleynik (1949): with total influenza morbidity rate figures of from 1000 to 1097 per 10,000 of the population in 1940-1946 the morbidity rate figures for children under the age of four ranged from 3680 to 9262.

The data of Ansheles (1945), who, apart from collecting statistical material for the city made a special collection of data on the actual morbidity rate in a district by means of the active detection of patients (Fig. 200), also attest to the higher morbidity rate in children compared with adults.

According to the data of Ritova (1953), the earliest age at which it has been possible to prove a case of influenza by laboratory methods was the age of 17 days; this occurred in an infant at home with its family, not in a lying-in home.

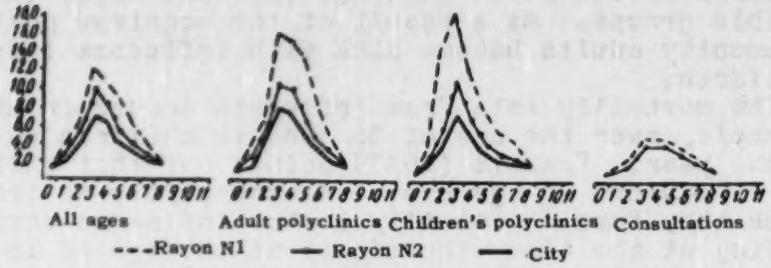

Fig. 200. Morbidity Rate Figures for Various Age Groups in Percentages (Ansheles, 1945).

In Fig. 201 data are presented concerning the influenza morbidity rate of different age groups compiled on the basis of a study of influenza in Moscow. In general, the material existing at our disposal on the age distribution of the influenza morbidity rate permits us to draw the following conclusion.

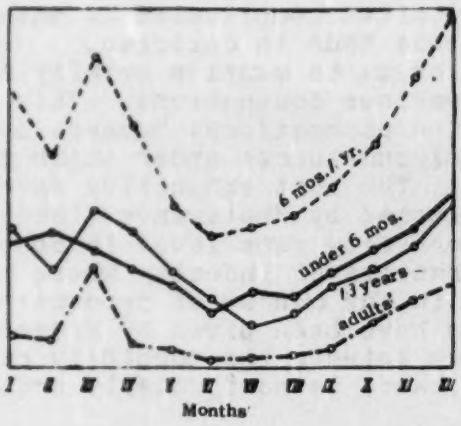

Fig. 201. Comparative Influenza and Acute Catarrh Morbidity Rate Curves Among Various Age Groups (in 1949).

Among children of pre-school age children become sick mostly between six months and three years of age; of these, the most vulnerable are those from 6 to 12 months.

Among school children the morbidity rate decreases with age, and children in the first three grades constitute the most susceptible groups. As a result of the acquired post-infectious immunity adults become sick with influenza less often than children.

The mortality rate from influenza occurs chiefly in older people, over the age of 55, and in children under the age of one year. Francis (1953) points out that children five to nine years of age are most susceptible to influenza. With time they form an immunity to the influenza strains circulating at the time; therefore, at the age of 15-24 years the morbidity rate drops sharply. A new rise in the morbidity rate at the age of 25-40 years is the result of the incomplete immunity formed in the past to the strains circulating at the time. Therefore, immunity is acquired as a result of new cases of the disease, which in its turn leads to a reduction in the morbidity rate in the age groups over 40. The age structure of the morbidity rate presented in Fig. 195 for 1918/19, 1928/29 and 1943/44 reflects the age immunological structure of the population. Older age groups rarely become sick with influenza, but they frequently develop pneumonic complications; the younger age groups frequently become sick with influenza, but it is rarely complicated by pneumonia in them; at the age of from 25 to 40 the influenza morbidity rate is moderate; influenza at this age is more often complicated by pneumonia but the mortality rate is less than in children.

It remains for us to mention briefly the connection of influenza with various occupations. This connection is not determined by the occupational hazards but rather by the conditions or circumstances under which the work is being accomplished. The most exhaustive data on this subject have been collected by Khotsyanov (1944), who showed that the highest morbidity rate level is observed among workers in those branches of industry where contact between people is greatest in the course of production (Table 99).

Similar data have been given by Zhdanov (1953) in which the difference between the morbidity rates in textile workers and coal workers is particularly pronounced (Table 100).

Table 99

Average Annual Number of Cases of Influenza per 100 Workers in the Most Important Branches of Industry in the USSR 1925-1939

Branch of Industry	No. of cases of influenza	Branch of Industry	No. of cases of influenza
Iron ore	13,8	Fur–leather	31,2
Coal	14,7	Printing	32,7
Petroleum	18,2	Transportation (trolley)	33,5
Ferrous metallurgy	18,7	Rubber	34,0
Paper	25,0	Tobacco	35,7
Plywood–sawing	25,5	Confectionery	37,0
Metal working	25,5	Sewing	37,5
Glass	26,0	Footwear	39,2
Textile	26,9		
Machine Construction	27,1	Average for all branches of industry	27,9
Cotton	27,2		

Table 100

Morbidity Rate and Work Loss From Influenza and Acute Catarrhs in Certain Branches of Industry, 1950-1952

Branch of Industry	Morbidity rate per 100 insured workers			Proportion of work loss to total work losses from diseases in percentages		
	1950	1951	1952	1950	1951	1952
Average data	67,6	60,5	87,3	6,9	5,8	8,5
Textile	102,0	89,8	136,4	12,0	10,3	15,0
Metallurgical	145,3	131,6	185,6	15,3	13,0	17,5
Coal	100,2	89,9	133,1	10,2	8,9	13,0

74. Seasonality and the Effect of Meteorological Factors

Grippe is one of those diseases which are not uncommonly associated with climatic conditions, with the weather, with meteorological factors. Its other name -- "influenza" -- which comes from the Italian expression "influenza di freddo," the effect of cold, is evidence of this. However, there are also opposite statements in the literature. Thus,

Nastyukov (1894) wrote that "the occurrence and spread of influenzal epidemics, and of influenza, does not depend either on the time of the year or on the change of weather or on a difference in climates." Hirsch (1885) adhered to a similar opinion. Statements of this kind may be encountered also in the more recent literature (Helfgat, 1940).

Nevertheless, the factual data do not permit us entirely to reject the importance of the environment (climate and meteorological conditions) in the epidemic process.

An analysis of statistical material shows that in the past 35 years all the influenza epidemics occurred during the cold season of the year. In the Soviet Union they have been observed from the end of October through April inclusive. In the United States according to the data of Collins (1945), out of 16 large epidemics recorded from 1920 through 1944 only one had its maximum mortality rate in the first week of May. All the others were observed in the winter time -- from November through March.

According to Hirsch (1881), out of 125 epidemics and pandemics of influenza 50 were in the winter (from December through February), 35 were in the spring (from March through May), 24 were in the autumn (from June through August). Vasil'yev (1889), noting that influenzal epidemics may occur at any time of the year and in any climate, points out that they occur most often in the winter and in the fall.

Certain authors (for example, Schaeffer and Shapiro, 1941) attempt to connect the development of influenza outbreak directly with severe cold.

Neklyudova (1952) compared data concerning the temperature, humidity, precipitation, direction and strength of the wind with data on the influenza morbidity rate in Krasmodar from 1943 through 1951.

Considerable rises in the influenza morbidity rate were noted during this period in the winter months of the following years: 1943-1944, 1945, 1946-1947, 1949-1950.

The increase in the influenza morbidity rate was always observed during the cold months; with the beginning of warm weather, with the rise in the air temperature, the morbidity rate decreased and remained lowest during the summer months. For the purpose of comparison data were used concerning the weather in the epidemic years and in years which were favorable with respect to influenza (1950-1951). There is a regular inverse relationship between the incidence of influenza and acute catarrhs of the upper respiratory tract and the outside air temperature, namely: with a reduction in the temperature there is always an increase in the morbidity rate, and vice versa. A certain relationship is found between the morbidity rate curve for influenza and the humidity. No distinct relationship was

found between the other indices (velocity of the wind, precipitation). However, the fact that there is an interrelationship between them can not be denied. The air temperature has an effect on the body along with the humidity, wind velocity and the atmospheric pressure.

A comparison of the meteorological data during the years of the epidemic rise in morbidity rate with the winter periods of 1947/48, 1948/49 and 1950/51, at which time there were no influenza outbreaks, shows that there is no marked difference in the weather variations between these years. It can be noted only that the greatest epidemic, in 1945, was observed in February, March, April, when the spring weather was somewhat delayed and the average air temperature in March was 0-1° and the precipitation increased to 90 millimeters. The second wave of the morbidity rate in this year occurred in December and January-February 1946. At the end of November the temperature dropped from 10° to 1°, and in the first few days of December it reached minus 5°. The increase in the morbidity rate accompanied the change in air temperature from hot to cold.

The fact should be noted that the absence of influenza A-prime epidemics in Krasnodar in the spring of 1949, that is during the period when it was observed in Moscow and in a number of other cities, coincided with the early advent of spring. The warming of the air in March reached 4-5°, on the average; in April, 10°; a small amount of precipitation, occasional winds and an abundance of sunny days were noted.

The rise in Influenza B morbidity rate in the winter of 1949/50 began in November when the air temperature dropped to 0° and reached -10° in December. At this time, the relative humidity was equal to 70-80 percent, the absolute humidity was equal to 15-20 millibars (a millibar is the unit of measurement of atmospheric pressure in meteorology, equal to 1/1000 bar), and the amount of precipitation reached 80 millimeters.

In Fig. 202 the curves are presented of the morbidity rate for influenza and acute catarrhs of the upper respiratory tract (according to a total record for 10,000 population). Then come the curves for the average monthly air temperature, the absolute and the relative humidity, the wind velocity, and the amount of precipitation.

From a comparison of the first curve with the subsequent curves it is seen that the air temperature drop corresponds, as a rule, to the increase in morbidity rate. A lesser degree of constancy of the relationships is shown through the examination of the indices for the air humidity. However, similar weather conditions occurred also during periods where there were no epidemic outbreaks of influenza.

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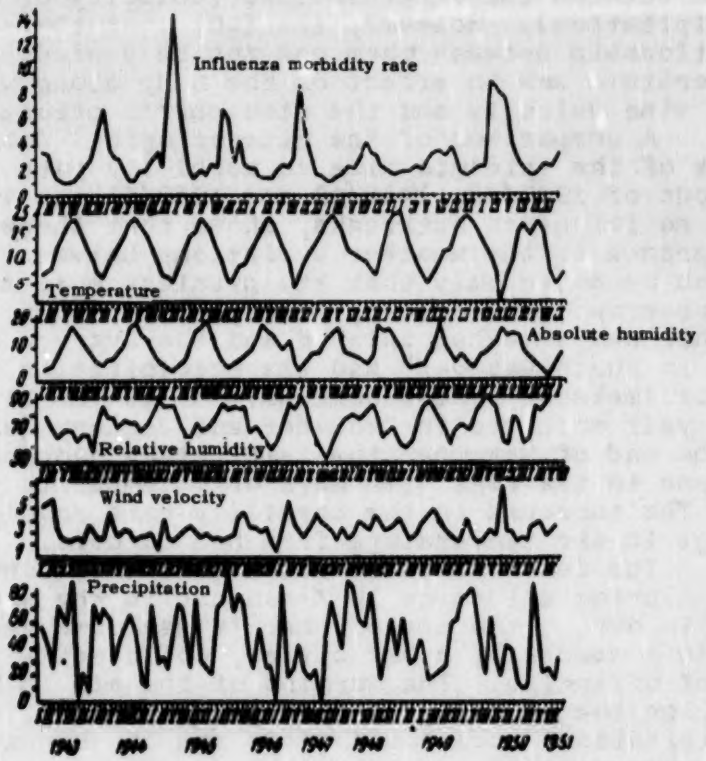


Fig. 202. Influenza Morbidity Rate and Meteorological Factors (Neklyudova, 1952).

In addition to Krasnodar we collected data concerning the weather during four epidemic outbreaks of influenza in Moscow: during the course of the preceding month and during the epidemic itself. These periods include the late autumn, the middle of winter and early spring: in 1943, November-December (influenza A); in 1946, January-February (influenza A); in 1946, November-December (influenza B); in 1949, February-March (influenza A-prime).

For the purpose of comparison, information is used concerning the weather in the winter season of 1944/45, at which time the cases of influenza were not of an epidemic nature: November-December 1944 and January-February 1945. Fluctuating cold weather, usual in the winter at the given latitude, is characteristic of all these periods.

Below, data are presented on two epidemics shown in

the form of graphs (Figs. 203, 204), where the increase in the influenza morbidity rate (the columns designate the daily figures) is compared with the result of meteorological observations.

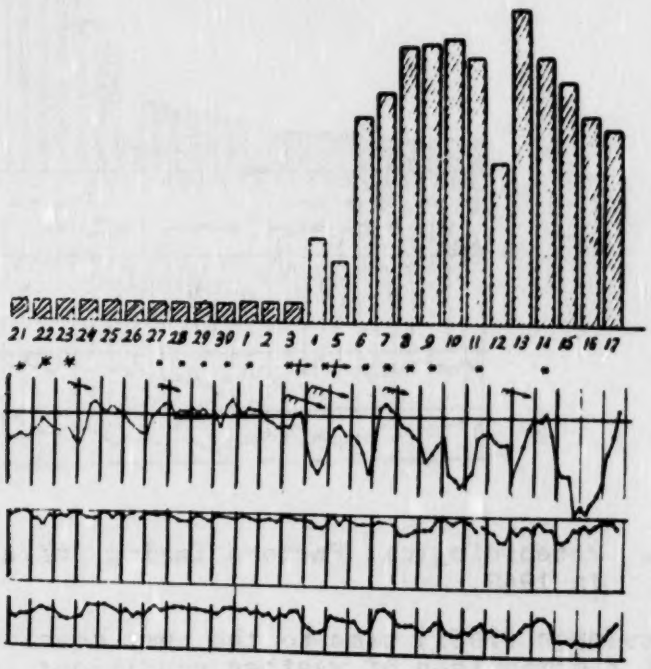


Fig. 203. Meteoroligical Factors During Influenza Outbreak in 1943.

The epidemics of 1946 and 1949 were accompanied by a reduction in the humidity. In contrast to them, the 1943 epidemic occurred in weather that did not have a dry frost period. The temperature variations were caused by marked changes in the direction of the wind. Despite the variance in the meteorological data, certain common conditions may be distinguished for all of the epidemics under analysis: 1) the presence of damp cold weather and fluctuating precipitations, zero temperature and a high degree of humidity; 2) severe cold; 3) considerable warming-up with copious precipitation and a strong South wind.

Therefore, all the influenza outbreaks listed occurred against the background of very much fluctuating weather. However, a comparison of these meteorological data

with the weather during the non-epidemic season 1944/45 showed that there is no essential difference between the years being compared with respect to the type of weather and its fluctuations.

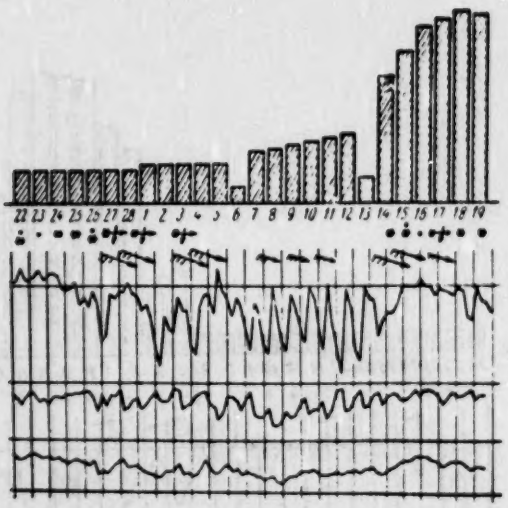


Fig. 204. Meteorological Factors During Influenza Outbreak in 1949.

Levinson (1951) came to the same conclusion as a result of a comparison of weather conditions and morbidity rate in Moscow and Tashkent. An analysis of the material taken for 12 years showed that a period of considerable fluctuation in temperature with a drop below 0° in combination with increased humidity and precipitation not uncommonly precedes and sometimes accompanies the influenza outbreaks. However, similar weather may occur also in the absence of any epidemic morbidity.

Zhilkina (1953) made an attempt to establish the influence of meteorological factors on the influenza morbidity rate in her work. Thus, in 1947 in a rural locality the increase in influenza morbidity rate in May occurred after a cold spell and considerable precipitation (Fig. 205). The author associates an influenza outbreak in a school in February 1952 (Fig. 206) with the same kind of meteorological influences. The author believes that sharp fluctuations in temperature and humidity reduce the body's resistance to influenza. There are a number of experimental observations in existence on the influence of the environmental tempera-

ture on the susceptibility of animals to influenzal infection. Thus, Saracino and Soul (1941) reported that heating and cooling of mice do not exert any noticeable effect in reducing the resistance of the animals. Based on this, they believe that influenza generally depends rather on the quantity and virulence of the virus than on the susceptibility of the host organism. Salkin (1945), as a result of his experiments found a difference in the susceptibility of mice to the PR8 virus depending on the temperature conditions at which they were kept. After showing that a temperature from 15° to 35° is harmless for them, he observed the development of influenzal pneumonia after the mice had been kept a week under these conditions before the infection. By comparison with control mice kept at a temperature 21-24°, less intense pulmonary pathology was found in the animals of the second group (kept at 35°) than in the animals of the first group which had been exposed to cooling, that is, kept at 15°. On the basis of these data a conclusion was drawn opposite to the conclusion of the former authors, to the effect that temperature conditions may exert an influence on the influenza outbreak by affecting both the infectious properties of the virus and the general resistance of the host.

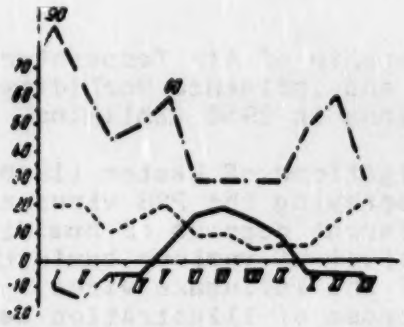


Fig. 205. Relationship of Air Temperature, Total Precipitation and Influenza Morbidity Rate in a Rural Locality in 1947 (Zhilkina, 1953).

Saslaw, Wilson, Doan, Woolpert and Schwab (1946) infected four rhesus monkeys by means of intranasal inoculation of the PR8 virus and kept them at a temperature of 4 to 6°. Two control monkeys which were under conditions of room temperature did not become sick after the infection. In the experimental animals a leukopenia, weakness and

respiratory disorders developed. Three monkeys died on the 6th, 11th and 17th days, from influenzal pneumonia, the authors believe. However, the defect in these investigations was the absence of a control wherein monkeys not infected with influenza would be kept at a low temperature. The latter makes the observations described less convincing.

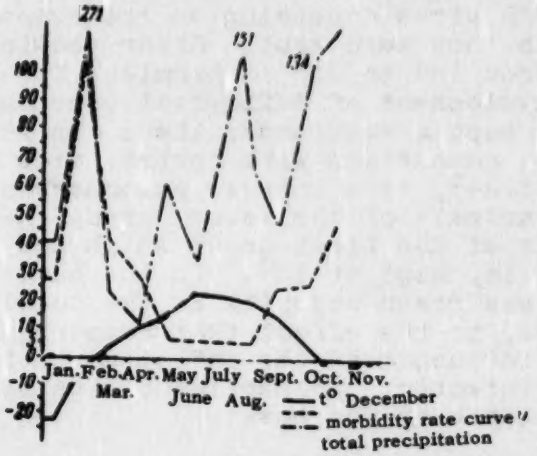


Fig. 206. Relationship of Air Temperature, Total Precipitation and Influenza Morbidity Rate under City Conditions in 1952 (Zhilkina, 1953).

The investigations of Lester (1948), who infected mice by means of spraying the PR8 virus and kept them in chambers with different degrees of humidity constitute evidence of the effect of various humidity conditions on the infectivity of the influenza virus.

For the purpose of illustration we are presenting a diagram which shows these experiments graphically (Fig. 207).

Everything which has been stated above attests to the fact that the effect of meteorological factors on the influenza morbidity rate can not be either excluded or overestimated. It should be emphasized that there is a need for distinguishing influenza from acute catarrhs of the upper respiratory tract. In the latter the weather and cold exert a direct effect on the body, whereas in influenza the meteorological factors of the environment affect the epidemic process chiefly because of a change in the contact conditions of people with one another. They contribute to or prevent the transmission of infection but are

all subordinate to various states of susceptibility of the population.

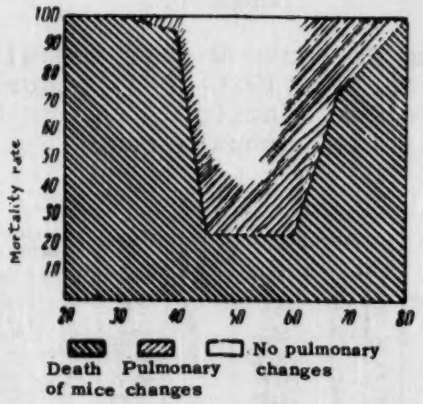


Fig. 207. The Effect of Relative Humidity on the Morbidity Rate and Mortality Rate From Virus Influenzal Infection (Lester, 1948).

We shall return to an explanation to the causes of the seasonal fluctuations in the influenza morbidity rate in the concluding part of this chapter. Here, we should like to note that, first, seasonal fluctuations in the influenza morbidity rate are indisputably established; secondly, they can not be explained by the effect of meteorological factors alone. The seasonality of influenza, as other infections with the air-droplet mechanism of transmission, is a complex phenomenon associated with a seasonal change in the life of people, variations in the susceptibility of the body and changes in the conditions of the virus in the environment. The first circumstance, apparently, is of decisive importance as a cause of the seasonal variations in the influenza morbidity rate.

75. The Epidemic Process and Characteristics of Influenza Foci

It has been mentioned repeatedly above that for the current epidemic process a constant influenza morbidity is characteristic throughout the year with seasonal variations and periodic outbreaks.

Numerous investigations prove the continuity of the epidemic process in large cities, where influenza is endemic and is encountered the year around. In generalizing on the

results of many years of investigations, Smorodintsev (1953) presents data on the constant circulation of the influenza virus among the population of Leningrad (Table 101).

Table 101

Type A Virus Among Patients With Diagnosis of Influenza in Various Epidemic Periods 1936-1941 (According to the Data of the Neutralization Reaction in White Mice With Type A Influenza Virus)

Year (Sept.-May)	Period of influenza outbreak	Types of virus	Interepidemic period		Pre-epidemic period		Epidemic period		Post-epidemic period		Total no. of patients investigated
			Total patients	Absolute no. of patients in whom type A influenza was found	Total patients	Absolute no. of patients in whom type A influenza was found	Total patients	Absolute no. of patients in whom type A influenza was found	Total patients	Absolute no. of patients in whom type A influenza was found	
1935/36	12/II–16/III 1936	A					26	22			26
1936/37	None	—	38	3							38
1937/38	10/II–30/III 1938	A	129	6	51	8	79	49	69	5	328
1938/39	20/I–20/III 1939	A	148	11	39	4	126	72	74	6	386
1939/40	20/III–30/IV 1940	B	270	10	87	2	(158)	6			515
1940/41	None	(?)	324	13							324
	Total . . .		909	43	177	14	388	149	143	11	1617

The proportion of cases of virus influenza among the diseases of the respiratory tract rises sharply during epidemic outbreaks and decreases in the interepidemic periods (Fig. 208).

Similar data have been obtained by the authors of this book; we made observations from 1946 through 1950 on the influenza morbidity rate in Khar'kov (Zhdanov and others, 1950) and in Moscow (Solov'yev and others, 1949). Apart from the statistical record of the morbidity rate, the polyclinics made serological and virological examinations by rayons and by districts.

During the course of this research it was shown that the influenza virus circulates among the population throughout the year. Its proportion with respect to the other

acute respiratory diseases decreases sharply during the summer and increases in the winter (Fig. 209).

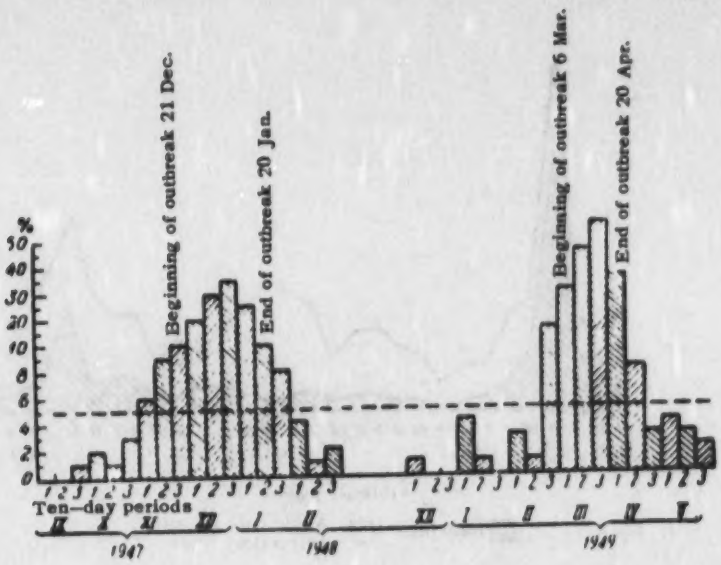


Fig. 208. Proportion of Influenza A Virus in Various Epidemic Periods 1947-1949 (Smorodintsev, 1953).

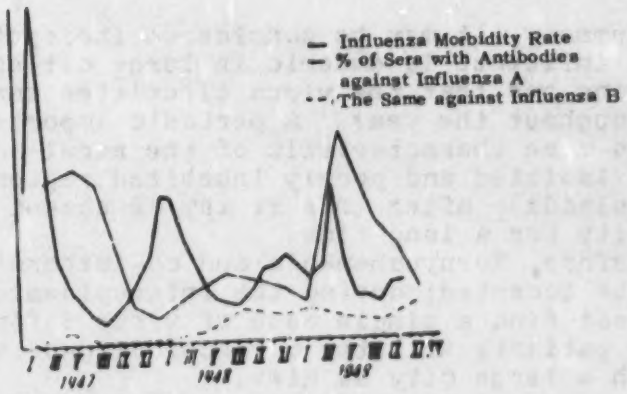


Fig. 209. The Relationship of the Influenza Morbidity Rate and the Data of Laboratory Investigations (Lukshina, 1950).

Similar data have been obtained by Dobrova (1954) in Moscow (Fig. 210).

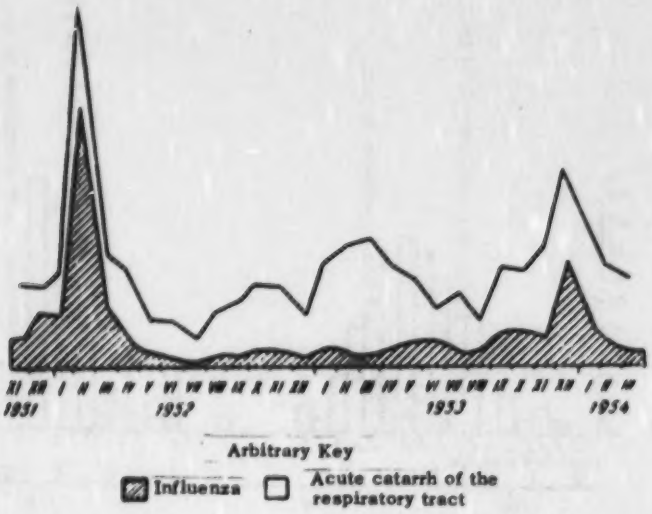


Fig. 210. Distribution of Total Morbidity Rate From Influenza and Acute Catarrhs According to the Data of Serological Study in 1951-1954 (Dobrova, 1954).

In summary, it may be considered indisputably established that influenza is endemic in large cities in the temperate zone and that the virus circulates continuously in them throughout the year. A periodic importation of influenza is more characteristic of the rural localities as well as for isolated and poorly inhabited regions (including small islands); after this it may be absent in the given locality for a long time.

Therefore, Kornyushenko's and co-authors' data (1953) can hardly be accepted; during the interepidemic period they could not find a single case of virus influenza among hundreds of patients with the clinical diagnosis of influenza in such a large city as Kiev.

The source of the infection in influenza is a sick person. Certain authors (Gromashevskiy and Vayndrakh, 1947) believe that there are no other sources of infection in influenza and that, specifically, "virus carriage by immune persons has no serious foundation as a hypothesis."

As a matter of fact, this is not so. Influenza is among the diseases in which the clinical picture varies

with respect to the degree of its expression and the severity of its course. Observations made during the pandemic of 1918-1919 speak for this also.

According to French's (1920) data, in the autumn in 1918 in England at the very height of the influenza pandemic, no clinical signs of the disease were observed in 800 out of every 1000 persons; in 180, the disease lasted three to five days and only in 20 did complications appear in the lungs, from which five to ten persons died. Modern investigations, as has been mentioned in the chapter on the clinical aspects, completely confirm the existence of abortive forms of the disease in influenza which border on asymptomatic infections, along with clinically overt cases.

The asymptomatic forms of influenza are recognized by many investigators at the present time and have been based on serological investigations and direct virological findings.

Francis and co-authors (1937), on the basis of serological investigations established the existence of a rise in antibody titer against the A virus in a number of people who did not have any clinical expressions of influenza.

Zhdanov and Korenblit (1949) expressed this idea on the basis of study of the influenza outbreak in 1947. Subsequently, the authors (Zhdanov and others, 1950; Lukshina, 1950) confirmed this idea with special observations. Below, their main results are presented.

In the study of the periodic rises in morbidity rate attention was directed to the lack of correlation between the statistical data for influenza morbidity and the frequency of occurrence of antihemagglutinins in the blood of those examined. In 1947, according to the data of polyclinics and health stations, throughout January, February and March about eight percent of the persons among whom the observations had been made became sick. If we take into consideration the fact that the percentage of persons who had antihemagglutinins in the blood increased from 6-8 to 21 during this time, it must be considered that influenzal infection included about 15 percent of the population. In February, March and April 1949 a new increase in the influenza morbidity rate was observed, at which time no more than three percent of the groups studied became sick. During this period the percentage of persons who showed a rise in antibody titer in the blood rose from 6 to 24, that is, it may be considered that the influenzal infection included about 18 percent of the population. Both these rises were considered to be influenza A. In January, February and March 1950 a rise in the influenza B morbidity rate occurred. The recorded morbidity rate amounted to 3.3 percent. The percentage of sera containing antihemagglutinins during this

period increased from 6 to 34, that is, by 28 percent. Therefore, throughout the three rises in influenza morbidity rate a notable predominance was noted every time in the frequency of occurrence of antihemagglutinins in the blood over the relative figures for those who had influenza.

At the beginning, this fact was treated as the result of an incomplete recording of the number of cases of influenza. However, there is every basis for the belief that the occurrence of antihemagglutinins against influenza is evidence of having had an asymptomatic infection. Considerable immunological shifts with respect to influenza among the population have occurred only after marked rises in the morbidity rate with respect to one type of influenza virus or another and have been absent in cases of cold catarrhs.

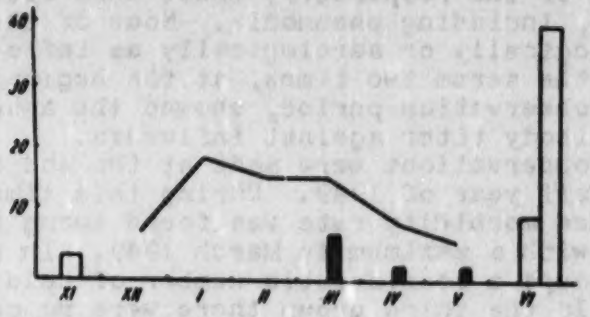
The considerable predominance of the frequency of occurrence of antihemagglutinins over the figures for the morbidity rate with the improvement in records for the latter has required a longer study of this phenomenon.

With this aim in view special investigations were made in groups in which a complete record and examination of the patients were provided.

One of the groups which was under observation consisted of men (217 persons). At the time of the initial examination of the sera in December 1948 antihemagglutinins against the influenza A virus were found in four persons. From December through May 75 cases of influenza were recorded in this group, of which the diagnosis of virus influenza A was established in eight cases. At the beginning of June, blood was again examined in the hemagglutination-inhibition reaction in 170 members of this group. Thereby, antihemagglutinins against the influenza A virus were found not only in six persons who had had influenza but also in 38 persons who had not sought medical aid for influenza and in whom there were no antibodies at the time of the first examination. The results of the examinations in this group are shown in Fig. 211.

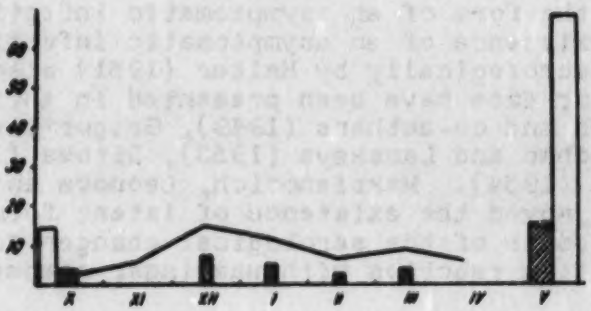
Another group consisted of adolescents from 11 to 17 years of age, and there were 400 persons in this group. On examination of the sera in October 1948, antihemagglutinins against the influenza A virus were found in 18 of them. During the period from October 1948 through April 1949, 15 cases of the virus type of influenza were found in this group and 47 cases with cold catarrhs. At the time of a second examination of the sera in May 1949 antibodies against influenza A were found not only in the 15 persons who had had virus influenza but also in 50 persons who had not sought medical aid during this period for influenza and in whom there were no antibodies found at the time of the first examination. The results of the examinations in this

group are presented in Fig. 212.



Influenza morbidity rate (hatched columns), morbidity rate with cold catarrhs (-), number of persons in whom antihemagglutinins were found in the sera ☐.

Fig. 211. Morbidity Rate From Influenza, Acute Catarrhs and Number of Persons in Whom Antibodies Were Found in the Group (Zhdanov and others, 1950).



Influenza (hatched columns) and cold catarrhs (-) morbidity rate; number of persons in whom antihemagglutinins were found in the sera ☐.

Fig. 212. Morbidity Rate From Influenza and Acute Catarrhs; Number of Persons in Whom Antibodies Were Found in the Group (Zhdanov and others, 1950).

In the third group, or more accurately, in a group consisting of smaller groups and in which there were 293 children from one and a half to five years of age, 85 cases of diseases of the respiratory tract were observed during this period, including pneumonia. None of them was diagnosed virologically or serologically as influenza. Examination of the serum two times, at the beginning and at the end of the observation period, showed the absence of any rise in antibody titer against influenza.

The observations were made at the end of 1948 and in the first-half year of 1949. During this time a rise in the influenza morbidity rate was found among the surrounding population with a maximum in March 1949. In all three of the main groups a considerable number of cold catarrhs was observed. In the third group there were no cases of influenza and there were no changes noted in the group immunity to influenza. In the first and second groups cases of influenza A were observed which coincided with the rise in influenza incidence among the surrounding population. Although only a small part of the members of these groups (about four percent) became sick with influenza, antihemagglutinins against the influenza A virus appeared in a greater number of persons (22 percent and 13 percent respectively) in comparatively high titers (1:160-1:320). These persons did not seek medical aid, and it was impossible to establish the fact that they had had influenza by retrospective interrogation.

The facts presented can have only one interpretation: these persons had had influenza in an extremely mild, latent form and in the form of an asymptomatic infection.

The existence of an asymptomatic infection has been established serologically by Kalter (1951) also.

Similar data have been presented in their works by Shimshelevich and co-authors (1949), Grigor'yev-Berenshteyn (1953), Klyachko and Lamskaya (1953), Ritova (1953), Orolova and Smil'gin (1954). Maksimovich, Leonova and Il'yina (1953) have proved the existence of latent forms of influenza on the basis of the serological changes as well as the hemagglutination reaction with washings, comparing these data.

There are also direct proofs in existence of healthy carriage (asymptomatic infection) in influenza. During the epidemics of influenza in 1943, Crowley, Thigpen and Rickard (1944) isolated the virus in 37 percent of the cases with clinically overt influenza and found a rise in the antibody titer in 86 percent. Simultaneously, healthy persons in whom no signs of disease were found were carefully examined in these foci.

Of the 13 persons examined in this way, the virus

was isolated from the washings of five; in nine a rise in antibody titer to influenza was found. It was also noted that in three persons who did not show any rise in antibody titer the virus was isolated from the washings.

Proofs of the existence of virus carriage in influenza have been obtained by Shishkina and Yurikas (1953). After examining 54 healthy persons who were in contact with influenza patients they isolated the B virus in six persons and in 21 established a rise in antibody titer against this virus by four or more times (Table 102).

Table 102

Examination of Healthy Persons Who Had Been in Contact With Patients With Type B Influenza

No. of patients in first five days of observation	No. of healthy persons in contact with patients	Of these No. who		Virus-carriers		Persons free of virus	
		had fever or became sick	did not become sick	Influenza type B virus found	rise of antibody titer by 4 or more times	virus-carriage not confirmed	Rise of antibody titer by 4 or more times
3	5	1	4	—	—	4	2
2	4	—	4	1	1	3	—
3	6	3	3	—	—	3	2
3	7	2	5	1	1	4	1
4	9	2	7	1	1	6	3
3	4	—	4	—	—	4	1
2	4	1	3	1	1	2	1
4	5	3	2	—	—	2	2
3	2	—	2	1	1	1	1
3	5	1	4	—	—	4	2
4	6	2	4	1	1	3	—
Total 34	57	15	42	6	6	36	15

Recently, Ritova (1954) succeeded in isolating the A-prime influenza virus from children who had been in contact with influenza patients, and Zakstel'skaya (1954) did the same from healthy persons, donors in blood transfusions stations. These findings were made during a period which preceded an influenza epidemic.

The facts presented cannot help but be of epidemiological significance. Observations made in groups permit us to express ourselves confidently on the existence of

latent and asymptomatic forms of influenza. Apparently, these forms of influenza develop in persons who have a relatively pronounced (partly lost) post-infectious immunity. The presence of latent and asymptomatic forms of influenza which are not uncommonly observed more often than the overt diseases explains the fact that the recorded morbidity rate is much less than the frequency of influenzal infection as established by means of mass immunological examinations. It may be supposed that these forms are of more than a little importance in the creation of group immunity to influenza. As far as the epidemiological significance of healthy carriage is concerned (asymptomatic infection) in influenza, this problem requires special study.

The incubation period in influenza usually does not exceed one or two days, which is evidenced by numerous old and new observations. Certain authors point to a more prolonged incubation period, of four or five days (Nagler and others, 1949). While this is possible, such a long incubation period is not very often encountered in influenza.

The effect of the short incubation period in influenza on the times at which other cases occur in foci is demonstrated distinctly by the data of Simpson (1941) presented in Fig. 213.

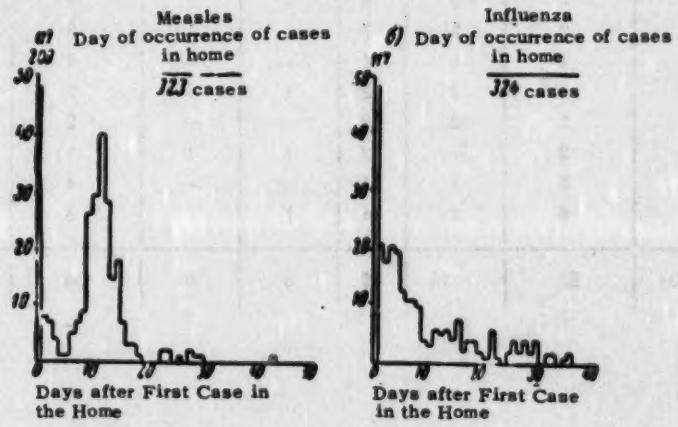

Measles
Day of occurrence of cases in home
123 cases
Days after First Case in the Home
Influenza
Day of occurrence of cases in home
Days after First Case in the Home

Fig. 213. Time of Occurrence of Other Cases in Measles and Influenza Foci (Simpson, 1941).

In measles the main mass of secondary cases occurs between the 10th and 14th day; in influenza, in the first five days after the onset of the disease.

The data presented show also the duration of the

contagious period in influenza. It is generally accepted that this period does not exceed five to seven days. The correctness of this conclusion is confirmed by data on the frequency of isolation of the influenza virus on various days of the disease, on the basis of results obtained through the work of the Commission on Acute Respiratory Diseases in the United States (1948) which are presented in Fig. 214.

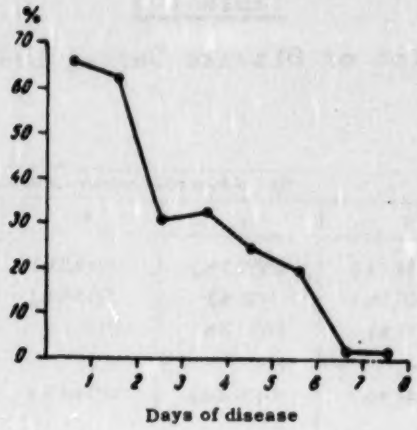


Fig. 214. Frequency of Isolation of Influenza A Virus From Patients (in percent) According to Days of the Disease (Commission on Acute Respiratory Diseases, 1948).

These data fully agree with the results of investigations made by Fadeyeva, Ritova, Zakstel'skaya, Slepushkin and Lozhkina as well as Marennikova and Gutman under the direction of the authors of this book (Zhdanov, Solov'yev) in the study of vaccine strains of influenza viruses. Since these data have been generalized in detail in chapters on variability and immunity, there is no need to return to them here.

Attempts have been made to connect the contagiousness of influenza, which is different for A and B viruses, with the different concentrations of the virus in the nasopharynx. Thus, for example, Hirst (1948) found an A virus concentration from nasopharyngeal washings of patients of 10^{-5}-10^{-6}, whereas the B virus could be found only in small quantities.

The frequency of multiple cases in foci is usually considerable. According to the data of Konova (1948),

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during an influenza outbreak in apartment houses where there were cases of influenza 50.7 percent of the residents became sick, and in families, 55.5 percent of the family members. Nine and four-tenths percent of the foci had a single case; 45.1 percent, had two; 30.4 percent, four or five; 15.1 percent, six or more. Similar data have been obtained by Shatrov (1948) in the study of the February outbreak of influenza in 1946 (Table 103).

Table 103

Focalization of Disease During 1946 Outbreak

No. of institution	No. of foci with number of cases				
	2	3	4	5 or more	total No. of foci
2	47(54,1%)	25(28,7%)	9(10,3%)	6(6,9%)	87
3	18(56,3%)	8(25%)	5(15,6%)	1(3,1%)	32
4	147(41%)	64(17,8%)	101(28,1%)	47(13,1%)	359
Total	212(44,4%)	97(20,3%)	115(24,1%)	54(11,2%)	478

During the interepidemic period the frequency of secondary cases in foci decreases markedly, which may be seen from Fig. 215 (Romanenko, Kaganskaya and Friedman, 1953).

Sergeyev (1950), confirming these observations, showed that the number of foci with secondary cases begins to increase from the start of the epidemic, at which time the statistical data still do not make it possible to establish the existence of the incipient outbreak.

A study of influenza and other diseases which have a similar clinical picture in families was made also by Dingle and others (1953). According to their data, children became sick most often; mothers became sick only half as often, and fathers only a fourth as much. Secondary cases in the families were observed chiefly in the four days following the first case.

For the purpose of characterizing the features of the epidemic process in influenza mention should be made of the investigations of Dobrova (1954) and Slepushkina (1954).

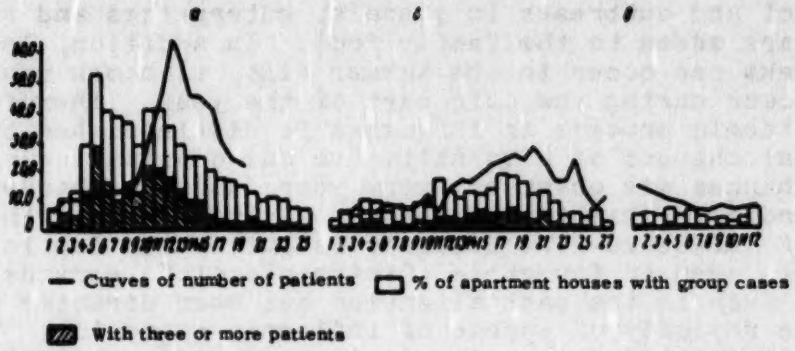

Fig. 215. Dynamics of Epidemic Process in Influenza and Focalization.

a -- influenzal wave in 1949; b -- wave in 1950-1951; c -- summer season (Romanenko and others, 1953).

For three years Dobrova has made observations on the influenza morbidity rate among workers in industrial enterprises who lived with their families in a village near the enterprise. The observations showed that family contacts play a great part in the spread of diseases. During the summer time, the diseases have the nature of isolated cases. With the advent of autumn the number of family-apartment house foci with several cases increases. During the epidemic outbreaks such foci account for the main mass of recorded cases of influenza. The predominance of family foci with multiple cases occurs before an outbreak is recorded statistically.

Slepushkina observed a similar picture in schools. Beginning with the first autumn month, the number of multiple cases in classes ("class foci") gradually increased, and with the advent of spring the number of them decreased. This was the case in 1952/53. In the following season (1953/54) outbreaks developed along with the class foci in the schools which included neighboring classes. Examination of the families showed that family foci occurred at the same time with multiple cases of influenza, and thereby the cases occurred more often in families first and then spread to the schools, although the reverse situation was also noted, importation of cases from school into the family. The observations described show that the epidemic process in a large city lasts for a whole year. In the summer, the

total number of cases and the number of family foci with multiple cases decreases. In the winter, they increase, and foci and outbreaks in schools, enterprises and institutions are added to the family foci. In addition, local outbreaks can occur in the summer also, although usually they occur during the cold part of the year. Therefore, the epidemic process in influenza is distinguished by seasonal changes of a quantitative and qualitative nature. Such changes are observed every year, and the observations made indicate that the proportion of influenza to the total mass of influenza-like diseases is quite high (up to 30-40 percent) even in favorable ("interepidemic") periods.

Even in the past attention has been directed to the extreme rapidity of spread of influenza epidemics. This has been pointed out particularly by Vasil'yev (1889).

Usually, epidemics of influenza A and A-prime are very short. According to the data of our many years of observations in Moscow and Khar'kov, even in such large cities the epidemics do not last for more than one and a half to two months. The correctness of this conclusion may be illustrated by the data presented above as well as by the examples of Mote and Loosli given in Figs. 216 and 217. Sadov (1932), referring to the data of Pearl, presents observations on the duration of influenza epidemics in 40 cities of North America: in 12 cities they lasted five to eight weeks; in nine cities, 9 to 12 weeks; in seven cities, 13 to 16 weeks; in six cities, 17 to 20 weeks; and only in three cities, more than 20 weeks.

Romanenko and co-authors (1950) present the following data on the duration and intensity of epidemics ("waves," according to his expression) of influenza in Leningrad (Table 104).

Table 104

Duration of "Waves" of Influenza in Leningrad

	Duration in weeks	Maximum degree to which the pre-epidemic level is exceeded
December 1943	8	16
January 1945	7	1,6
February 1946	6	12
November 1946	4	5
December 1947	4	4

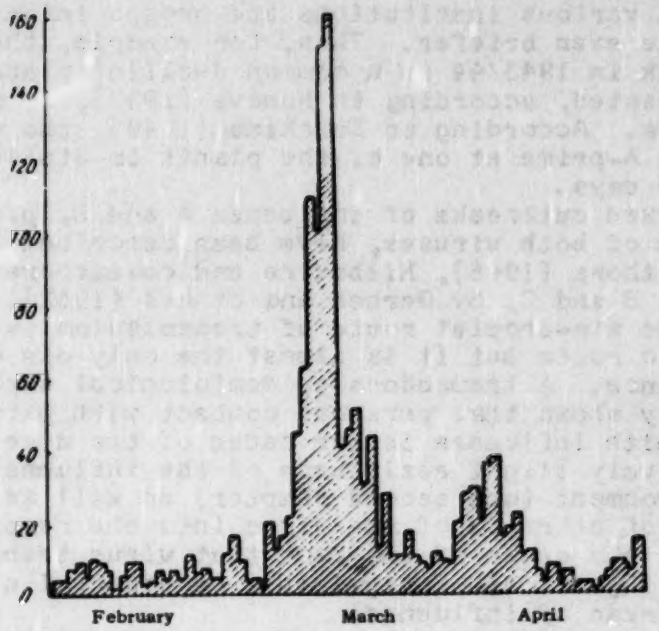

Fig. 216. Influenza Epidemic in American Military Camp, Funston, in the Spring of 1918 According to the Data of Daily Sick List (Mote, 1943).

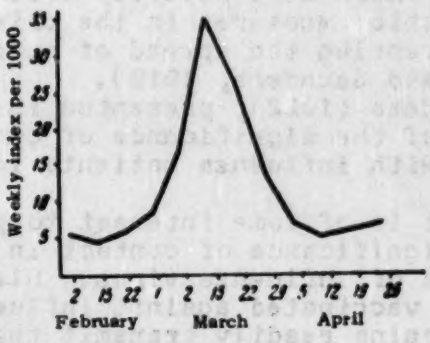

Fig. 217. Morbidity Rate According to the Hospitalization Data During an Influenza Epidemic in 1947 in Chicago (Loosli and other, 1948).

At various institutions and groups influenza outbreaks are even briefer. Thus, for example, the influenza A outbreak in 1943/44 in a common dwelling place of a single combine lasted, according to Konova (1948), no more than 35-40 days. According to Dubakina (1949), the outbreak of influenza A-prime at one of the plants in Stalingrad in 1949 lasted 40 days.

Mixed outbreaks of influenza A and B, proved by the isolation of both viruses, have been described by Burnet and co-authors (1946), Kilbourne and co-authors (1951); of influenza B and C, by Gerber and others (1952).

The air-droplet route of transmission is not the only basic route but it is almost the only one of practical significance. A tremendous epidemiological experience has invariably shown that personal contact with patients (infected) with influenza is the cause of the disease. The comparatively slight resistance of the influenza virus in the environment (see second chapter) as well as the inevitability of entrance of the virus into the respiratory tract are the cause of the fact that virus transmission by means of objects is of negligible or doubtful significance in the spread of influenza.

It is specifically for this reason that, where it can be accomplished, isolation makes the spread of influenza practically impossible. During the influenza pandemic in England out of 415 inhabitants of 71 lighthouses only eight inhabitants of four lighthouses became sick, 20 times less than the average morbidity rate for the main population of the country. During this epidemic, 21 German and 39 English prisons remained untouched by influenza, although some of them were in foci which were severely affected (Sadov, 1939). The simplest isolation measures in the prisons produced a rapid effect, preventing the spread of cases among the prisoners (Smith and Saunders, 1919).

Vaughan's data (1922), presented in Table 105, constitute evidence of the significance of contact, that is, living together, with influenza patients in the occurrence of the disease.

Finally, it is of some interest to present our own material on the significance of contact in the transmission of vaccine strains of influenza virus. Slepushkin (1954) showed that those vaccinated against influenza with man-adapted tissue strains readily transmit the virus to contacts if the latter live in the same room. However, such a transmission occurs comparatively rarely in co-operative work at a factory if there is no crowding of the workers in the shops. Apparently, the dose of virus which enters the respiratory tract is far from having the last say in the outcome of the infection.

Table 105

Influence of Degree of Contact With Patients on the Influenza Morbidity Rate

Nature of contact with patients	No. of persons subject to risk of infection under these conditions	No. of those who became sick	Morbidity rate
Same bed	360	166	45.2
Same room	303	59	19.5
Same apartment house	1 064	273	22.3

Although the air-droplet route of transmission is the main one and almost the only one in the epidemiology of influenza, under certain conditions the household articles, chiefly utensils, can play a certain part in the spread of the disease. Lynch and Cumming (1920) present observations on 66,000 soldiers in 11 camps: boiling of the utensils decreased the morbidity rate and the mortality rate among the soldiers by three to five times (the morbidity rate figures per thousand were, respectively, 252 and 51).

In the preceding section data have been presented concerning the influence of meteorological factors on the spread of influenza. Here, it is fitting to supplement them with certain data concerning the spread of influenza in various latitudes of the USSR.

In Table 106 and in Figs. 218-220 data are presented concerning the course of influenza A-prime epidemics in 1949 and 1952 in different localities of the USSR. In the first column of the Table the average monthly morbidity rate is shown for June and July; in subsequent columns the figures show by how many times and in which months this morbidity rate level was exceeded.

An analysis of these data permits us to draw two conclusions. First, influenza epidemics in the northern latitudes develop before those in the southern latitudes.

Secondly, the sharpness of the rises in influenza morbidity rate in the southern latitudes is greater than in the northern latitudes, where even in the summer a comparatively high level of influenza morbidity rate is observed.

Table 106

Development of A-Prime Influenza Epidemics in 1949 and 1952 in Certain Cities of the USSR

No. of city	City	Aberage monthly morbidity rate for July–August 1949/50	1949			1952		
			February	March	April	February	March	April
1	Moscow	40 826	2	8,5	4	13	6	3
2	Leningrad	22 322	2	9	6	7	19,5	4
3	Gor'kiy	8 109	1	1	2,5	3,5	6,5	2,5
4	Kuybyshev	2 791	2,5	5	2,5	5,5	14,5	4,5
5	Saratov	3 907	1,5	2	2	2,5	7,5	3
6	Stalingrad	1 647	1,5	2,5	6	3	11	4
7	Tbilisi	149	14	15	19	12	45,5	12
8	Yerevan	205	7,5	18	6	3,5	10	5
9	Baku	4 832	3	3	4	2	8	4
10	Stalinabad	367	2	1	3	1,5	2,5	2
11	Tashkent	2 019	1	1	1,5	2,5	2,5	2
12	Ashkhabad	184	3	4	5	3	2,5	3
13	Alma-Ata	1 062	2	3	3	4	7,5	2
14	Frunze	24	2	3,5	2	66	275	72
15	Sverdlovsk	5 204	1,5	4	1,5	11,5	4	2
16	Perm'	3 866	1	3	2	4	5	1,5
17	Novosibirsk	1 917	2	6,5	5	29	7	5
18	Krasnoyarsk	280	No information			44	6	2,5
19	Kiev	1 394	5	4	6	7	23,5	13,5
20	Rostov-na-Donu	4 894	2,5	4	4,5	3	8,5	4

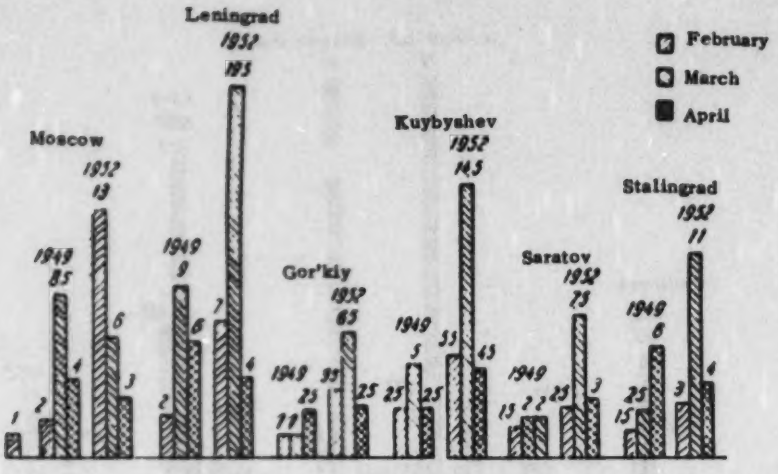

Fig. 218. Development of Influenza Epidemics of 1949 and 1952 in Certain Cities of the USSR.

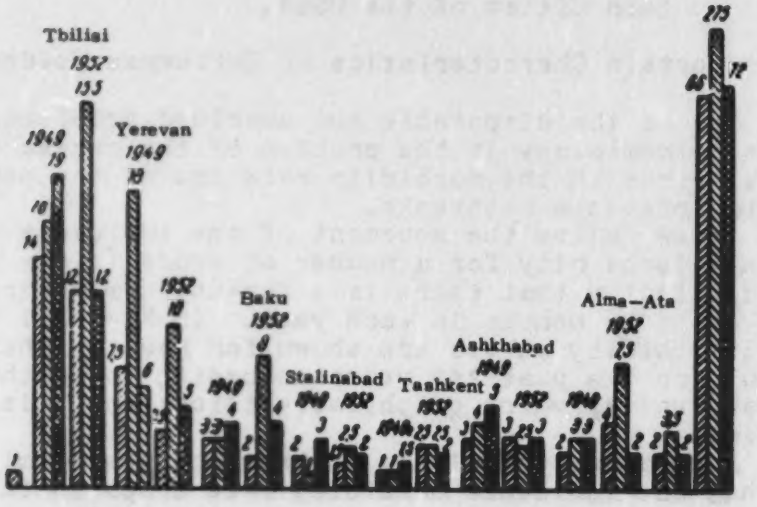

Fig. 219. Development of Influenza 1949 and 1952 Epidemics in Certain Cities of the USSR.

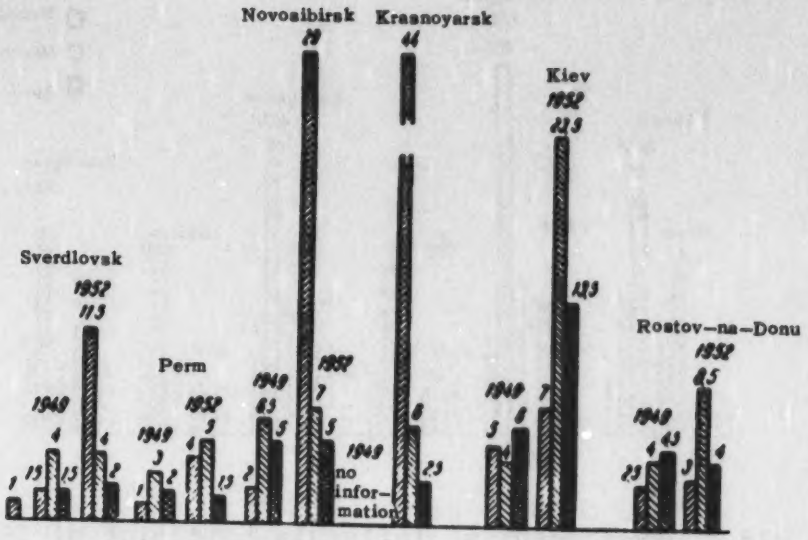

Fig. 220. Development of 1949 and 1952 Epidemics in Certain Cities of the USSR.

76. Certain Characteristics of Influenza Epidemiology

One of the disputable and unsolved problems of influenza epidemiology is the problem of the causes of seasonal rises in the morbidity rate and of the periodic epidemic influenza outbreaks.

If we follow the movement of the influenza morbidity rate in a large city for a number of years it may be shown quite distinctly that there is a non-uniform distribution of the cases by months in each year. In Fig. 221 the influenza morbidity curves are shown for Moscow, Khar'kov and Tashkent for the past ten years according to months in arbitrary units, which graphically illustrate this characteristic.

Even a cursory glance at these curves permits us to note that the influenza morbidity rate drops in the spring months, reaches a minimum in the summer and increases in the autumn months, reaching a maximum in the autumn, winter or spring. With a more careful study of them it may be seen that the influenza morbidity rate rises in the cold months are different in different years.

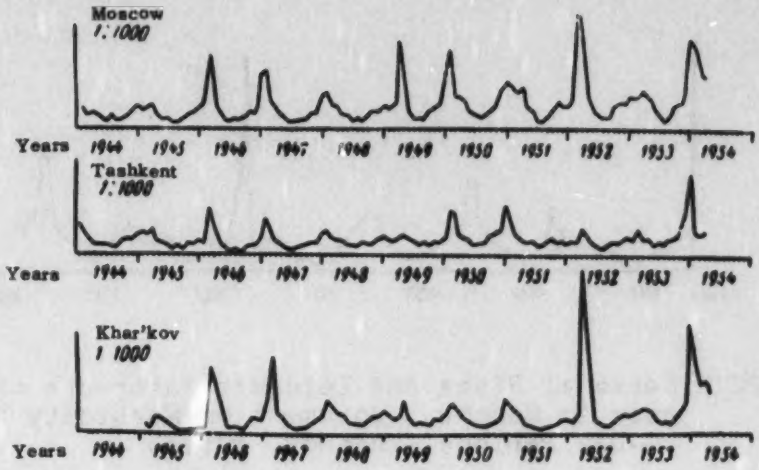

Fig. 221. Influenza Morbidity Rate From 1944 Through 1954 by Months in Moscow, Khar'kov and Tashkent (Zhdanov, 1954).

Let us analyze the movement of the influenza morbidity in Moscow from July 1948 through May 1954 (Fig. 222). During these years the rises in the morbidity rate during the cold season of the year were of two types. In 1950/51 and 1952/53 the morbidity rate increased gradually, beginning with September, reached maximum figures in January, after which it just as gradually decreased. In 1948/49, 1949/50, 1951/52 and 1953/54 brief but active epidemic outbreaks occurred against the background of a gradual increase in the morbidity rate; the maximum development of these outbreaks occurred in different months: in 1949, in March-April; in 1949/50, in December-January; in 1952, in February-March; and in 1953, in December.

Returning to the curves shown in Fig. 221, it may be seen that these two types of rises in influenza morbidity rate occur in all three cities.

Before drawing any final conclusions, however, concerning the types of epidemic process it is necessary to clarify the degree to which these conclusions may be applied to influenza, because the curves presented above reflect the sum of diseases diagnosed clinically as influenza and as acute catarrhs of the respiratory tract.

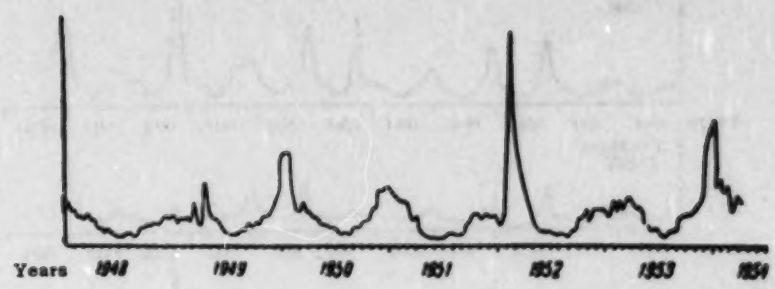

Fig. 222. Seasonal Rises and Epidemic Outbreaks of Influenza in Moscow. Movement of Morbidity Rate by 10-Day Periods (Zhdanov, 1954).

For a proper evaluation of the etiologic structure of the cases the results of mass serological investigations of patients with diagnoses of influenza and acute catarrhs of the respiratory tract are of importance, as are also data concerning the influenza viruses isolated during these years. These investigations have shown that virus influenza in the summer months does not comprise more than 10-15 percent of the cases which go under the clinical diagnoses of influenza and acute catarrhs; during the autumn-winter rises its proportion goes up to 30-60 percent; and during the epidemic outbreaks, up to 80-90 percent. A more detailed study of the etiologic structure of diseases in Moscow showed that before 1949 during the periods of both increase and decrease in the influenza morbidity rate A viruses (more often) and B viruses (less often) were isolated from the patients, and beginning with 1949 the A virus stopped being recovered and it was replaced by the A-prime virus. As far as the epidemic outbreaks are concerned, each of them was associated with some single influenza virus. Of the last five outbreaks in Moscow one (1947) was associated with the A virus, one (1949-1950) with the B virus, and three (1949, 1952 and 1953) with the A-prime virus.

Therefore, the epidemic process in influenza occurs in two forms: in the form of seasonal variations in the morbidity rate which are of moderate intensity, and in the form of brief intense epidemic outbreaks. The third form, which is encountered much less often, is constituted by the influenza pandemics. They were analyzed in greater detail at the beginning of this chapter. Naturally influenza

pandemics are characterized not only by the intensity of the morbidity rate, which goes beyond the realm of ordinary epidemics, but also by their spread throughout the entire world.

These types of epidemic process have been noted, although not entirely accurately, by other investigators also.

Even in the past century the distinctness of the development of epidemics and pandemics of influenza has been noted. For example, Hirsch (1881), in describing certain epidemics, points out the lines of spread of diseases, and in certain cases even the place of their onset.

On the other hand, Vasil'yev (1889) points out that many epidemics of influenza have no definite trend and not uncommonly begin simultaneously in opposite parts of the world.

Sadov (1932) notes that the epidemic of influenza spreads through a locality like a spot of oil, and in localities where the lines of communication are poorly developed it moves slowly from village to village. At the same time, according to the data of Dopter, sometimes influenza epidemics appear simultaneously in different cities.

In analyzing the influenza A-prime epidemics, Andrewes (1951) concludes that the method of spread of influenza epidemics is not clear. In 1949, an epidemic began in Sardinia, spread throughout Europe and reached Iceland, that is, it spread from country to country. In 1950-1951, the development of epidemics was completely different: the summer outbreaks in various countries grew into an epidemic in the autumn, that is, in his opinion, activation of the virus occurred simultaneously in different countries.

American authors point out that influenza A occurs periodically every two or three years; influenza B, every four-six years. However, Stuart-Harris (1947) did not observe such a system in England.

Stuart-Harris (1947) notes three types of annual influenza morbidity rate in the large cities of England: sharp rises with a mortality rate of more than a thousand persons a week; irregular protracted rises with a mortality rate of more than 100, but not more than 200-400 a week; and rises with a low mortality rate, less than 100.

Romanenko (1952, 1953) expresses his viewpoint concerning the existence of two types of influenza waves: those with a high morbidity rate level and brief, low-level morbidity-rate waves which, however, drag out for three to five months. In contrast to the other infections a sharp fall in morbidity rate occurs in influenza after the epidemic outbreaks.

The seasonal variations in influenza morbidity rate are customarily associated with meteorological factors. This explanation suggests itself, because from year to year the minima of the morbidity rate occur in the warmest months of the year (June-July), while the maxima, in the coldest months of the year (December-January). However, a more attentive study of the problem has shown the worthlessness of this explanation. First, the increase in the influenza morbidity rate begins everywhere starting with September, when the weather is still warm in the temperate and in the tropical areas; secondly, the decrease in the morbidity rate begins in February and sometimes in January, that is, at the height of the cold weather; thirdly, the cause of occurrence of epidemic outbreaks in different months -- from April (1949) to December (1953) is absolutely incomprehensible. Without denying the effect of the meteorological factor on the epidemic process in influenza (more will be said about this below), it should be recognized, nevertheless, that it is not this factor which determines the characteristics of the epidemic process mentioned. Attempts have been made to connect the seasonal variations in the influenza morbidity rate also with changes in the living conditions of people at different times of the year. Thus, the September rise in influenza is associated with the opening of school after vacation.

At this time, the proportion of school children among those who become sick with influenza actually does increase. In the winter, there is a closer contact of people in rooms, which increases the possibility of spread of influenza. However, this explanation does not permit us to understand why the influenza morbidity rate begins to fall even in February, and it does not even begin to answer the question of the causes of epidemic outbreaks of influenza.

The causes of the seasonal variations in the morbidity rate and the periodic epidemics not uncommonly are sought in the fluctuations of immunity in the population. This explanation aids in understanding certain characteristics of influenza epidemiology; particularly the fact of the reduction of influenza morbidity rate in the middle of the winter, at which time there are all the conditions present favoring the spread of the infection, is in agreement with it.

In general, the decrease in immunity to influenza among the population usually precedes the occurrence of an influenza outbreak. The antibody titers may serve as a definite index of the state of immunity. Thus, for example, Vasil'yeva, at the suggestion of Smorodintsev (1943), investigated the antibody titer against A and B viruses in

the population (donors were investigated), and this level rose sharply after the epidemic (Fig. 223). Similar data have been presented in the work of Zhdanov and Korenblit (1949), who observed the influenza morbidity rate in Khar'kov in 1947 (Fig. 224).

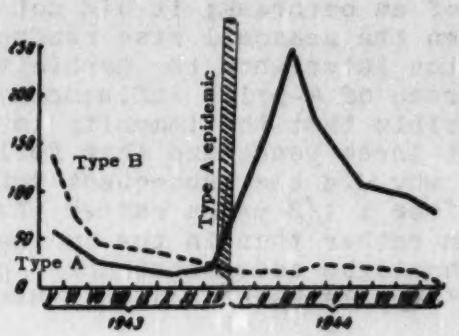

Fig. 223. Change in the Average Figures for Antibody Titers Against Influenza A and B Viruses in a Constant Population Group of 200 Persons (Smorodintsev, 1953).

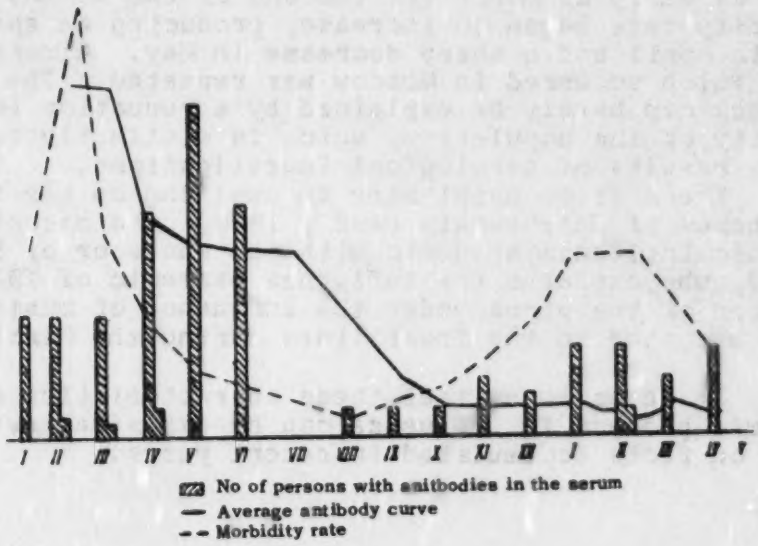

Fig. 224. Dynamics of Influenzal Diseases and Dynamics of Increase in the Antibody Titer (Zhdanov and Korenblit, 1949).

The cause of the epidemic outbreaks of influenza which occur from time to time, however, remains unclear, which may be shown through the example of outbreaks of this disease in Moscow.

In March-April 1949 an outbreak of A-prime influenza occurred. After this almost three years passed during which the routine seasonal rises in morbidity rate did not lead to the development of an outbreak; it did not even develop in December 1951 when the seasonal rise reached a maximum. However, two months later when the morbidity rate had dropped, an outbreak of A-prime influenza suddenly occurred. Is it really possible that the immunity in the population lasted for almost three years and then fell off sharply in two months? And why did the subsequent influenza A-prime outbreak occur after 1 1/2 years rather than after three, and in the autumn rather than in the spring? To what has been stated it should be added that these phenomena were repeated in Khar'kov, Tashkent and in a number of other cities.

In Fig. 225 data are shown concerning the influenza morbidity rate in Khar'kov in 1948/49. As is seen, a slight winter rise in morbidity rate occurred from November through January with a low antibody titer among the population. The morbidity rate began to fall off in January and decreased still further in February, which was documented also by virological and serological investigations. However, as early as March (at the end of the month) the morbidity rate began to increase, producing an epidemic rise in April and a sharp decrease in May. Almost the same thing which occurred in Moscow was repeated. The April outbreak can hardly be explained by a reduction in the immunity of the population, which is distinctly seen also in the results of serological investigations.

There is no point here in dwelling on the fantastic hypotheses of Chizhevskiy (1927, 1930), who associates the periodic influenza epidemic with sun spots or of Richardson (1948), who explains the influenza pandemic of 1918/19 by a mutation of the virus under the influence of mustard gas which was used in the front lines during the First World War.

It seems to us that these characteristics of the epidemic process in influenza can be explained rationally, based on facts accumulated in recent years.

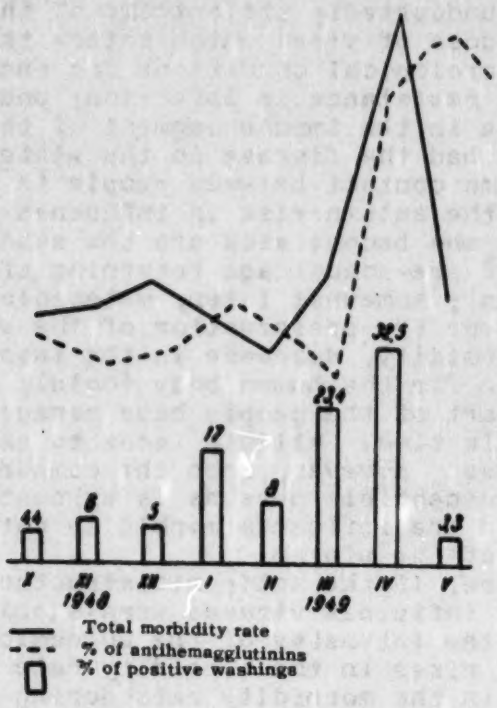

Fig. 225. Seasonal Rise in Influenza A in December 1948 and During Epidemic of Influenza A-prime in April 1949 in Khar'kov (Zhdanov and others, 1950).

After an epidemic outbreak of influenza which includes a considerable part of the population a strongly immune segment is formed which makes any repetition of an influenza epidemic caused by the same virus impossible, at least in the next few years. Circulation of the virus, however, continues, because by far not everyone has influenza (or has the infection in an asymptomatic form), not to mention the fact that children who are born and growing children as well as those coming into a large city form a constantly increasing reservoir of persons susceptible to influenza. This reservoir is also filled by persons who have gradually lost their immunity acquired as a result of the infection which they had had. In the summer time, the intensity of the epidemic process slackens for a number of reasons: less contact between people in closed rooms, less

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favorable meteorological conditions for preservation of the virus in air (undoubtedly the outcome of the infection must depend on the dose of virus which enters the body), more favorable meteorological conditions for the human body, increasing its resistance to infection; and finally, because of the increase in the immune segment of the population from those who have had the disease in the winter time. With the advent of autumn contact between people in closed rooms increases (in the autumn rise in influenza morbidity rate the first ones who become sick are the school children and the children of pre-school age returning after their summer health campaign); somewhat later, meteorological changes occur which favor the preservation of the virus in air (increase in humidity, decrease in the insolation) which are unfavorable for the human body (cold); moreover, a considerable part of the people have managed to lose their immunity by this time. All this leads to an increase in the epidemic process. However, soon the comparatively small reservoir of susceptible persons is exhausted; therefore, the decrease in the influenza morbidity rate usually begins in the middle of the winter.

Therefore, if the antigenic structure (immunological properties) of influenza viruses were stable, seasonal variations in the intensity of the epidemic process -- the alternation of rises in the morbidity rate during the cold and decreases in the morbidity rate during the warm part of the year -- would be the only type of epidemic process in influenza. Actually, in the period between the epidemic outbreaks and under large city conditions these variations are of quite a constant nature, in connection with which the curves of the influenza morbidity rate during the "calm" years are quite unitypical.

The antigenic structure of influenza viruses, however, from year to year, changes under the influence of the specific immunity of the population. This occurs because in influenza, in contrast, for example, to measles the immunity weakens quite rapidly, and the influenza virus, after entering a partially immune body, can multiply in it, producing an asymptomatic infection in the majority of cases and disease only when there is a considerable loss of immunity. In summary, the influenza virus multiplies more often in the immune than in the non-immune organism and, adapting itself to new environmental conditions and changing, it overcomes its specific immunity. When these changes lead to the occurrence of a qualitatively new antigenic variety of the virus which readily overcomes the specific immunity of the population to the old strains, an epidemic outbreak of influenza may develop under certain conditions, produced by the new variety of virus. Naturally, the formation of a

new antigenic variety of the virus depends on a multitude of conditions and cannot occur at regular periods; therefore, the intervals between the epidemic outbreaks are different, particularly since the new variety of virus which occurs does not always meet with favorable conditions for its spread. Since the formation of a new variety is most probable at the height of the epidemic process, at which time the circulation of the virus among the population occurs most actively, one cannot be surprised that the epidemic outbreak almost always occurs during a maximum in the seasonal rise in morbidity rate or after it. The appearance of new varieties occurs at first by means of the cultivation of new antigenic groupings with a maintenance of the old antigens for a certain time; therefore, the immunity to the new varieties for a certain time includes the immunity to the old strain. This explains, first, the gradual disappearance of the old virus varieties and the appearance of new (for example, the gradual disappearance of virus A and the appearance of A-prime) ones, and, secondly, the appearance of antibodies against the old strains in people after infection with new strains (for example, the occurrence of antibodies against influenza A in a case of influenza A-prime).

Omitting an analysis of the factual material which provides a basis for the considerations stated above, about which we have already spoken, let us compare only certain facts which pertain to epidemic outbreaks of influenza A and A-prime during the last five years.

As has already been mentioned, the usual seasonal course of the epidemic process in 1948-1949 was interrupted by a large-scale epidemic outbreak of influenza. It is very characteristic that before the onset of this outbreak at the height of the seasonal rise in morbidity rate A viruses which were common at that time were isolated from the patients; during the outbreak, strains of a different antigenic structure were isolated from the patients which were later identified as A-prime virus strains. The 1952 outbreak was produced by A-prime viruses; however, in their antigenic structure they were notably different from the 1949 strains, which gave Gorbunova the basis for separating them out into a separate variety of A-prime virus. The same thing also occurred in 1953, at which time Ritova managed to isolate strains of A-prime virus which were different in their antigenic structure from the 1952 strains. It is a very curious fact that this last variety of A-prime virus was found for the first time in the summer of 1953 during a considerable local outbreak in a relatively isolated group. The summer did not favor the spread of the new virus variety much among the population; on the other

hand, the epidemic outbreak in that year occurred unusually early in the city, in the autumn rather than in the spring or winter, as it usually occurs.

On the basis of the facts accumulated we have attempted to explain certain rules and regulations in the epidemiology of influenza under conditions of a large city. It goes without saying that the considerations presented to a certain degree schematize the epidemic process, which is more complex, because in various regions of the USSR local characteristics may be observed in the epidemiology of influenza in any city of the Soviet Union, particularly in Moscow.

Other investigators agree with the explanation presented. Kondrat'yev (1953), on the basis of an analysis of the influenza epidemics of the last three decades, concludes that influenza outbreaks are created from local strains which have changed their antigenic structure under the influence of immune forces of the body. Smorodintsev (1953) comes to the same conclusion in his recent works.

It remains for us to analyze the last problem -- the causes of development of influenza pandemics. A comparison of the data presented here and that at the beginning of the chapter shows that there is not only a quantitative difference between epidemics and pandemics of influenza, as is mentioned by Gromashevskiy (1949), who calls a pandemic "an unusually severe epidemic which in its intensity considerably exceeds that of epidemics which are usual for a given disease, given locality and given historical conditions." Influenza pandemics are distinguished from epidemics primarily by essential qualitative characteristics. While the epidemics include one or a few economically associated countries, as has been shown, influenza pandemics always have a world-wide distribution. Whereas epidemics occur in various countries at different times and the synchrony of the morbidity rate is limited usually to one or several countries, and in the USSR influenza epidemics usually do not even include the entire territory of the country (Fig. 221), pandemics develop throughout the world.

Here, several years are usually required for the development of a pandemic, as occurred in 1918/19 and in 1947-1952, rather than one year.

It seems possible to us to consider the development of pandemics as a world-wide spread of a new virus (type or variety) of influenza, whereas an epidemic is a process whereby it circulates within a certain locality (country, city). The completion of the spreading process of the new virus signifies the end of the pandemic. It is specifically through this difference between the pandemic and the epidemic that the qualitative characteristics of influenza A-

prime epidemics in 1947-1949 (spread throughout a territory) in contrast to epidemics of it in 1950-1951 (development of epidemics in Western Europe in the absence of them in the United States and USSR). This feature has been noted but not explained by Andrewes (1951), who described two types of spread of influenza epidemics. The manner in which he treated this phenomenon together with Isaacs (1951), attempting to find the causes of this phenomenon in the P-Q-variants of the virus, were not entirely erroneous.

Of the influenza pandemics described in the literature the last one was the most carefully investigated (1947-1952). We have attempted to show that the variability of the A influenza virus, which occurred in approximately the same direction throughout the world, led to the formation of the A-prime virus between 1945 and 1947. Apparently, the final development of this virus took place in the United States, from where it began its spread, which resulted in its taking root in the entire world in 1949. This process was completed in 1952. In the same year, the last influenza pandemic was concluded.

The numerous and heterogeneous facts presented in this and in preceding chapters make it possible to reconstruct the general course of the development of the 1918/19 pandemic. It may be supposed that the first influenza wave was associated with an active spread of the old virus. After a prolonged separation of connections between countries, the intensified relations between countries at the end of the First World War led to the development of epidemics in the majority of the countries of the world and to a considerable increase in the immunity of the population. The strong immunity which rapidly occurred was a factor in changing the antigenic structure of the virus, as a result of which a new virus of the swine influenza type was formed. Apparently, this process was completed in the second half of 1918, after which the spread of this new virus began throughout the world, that is, a pandemic of "Spanish" influenza. Since summer came soon afterwards this process was somewhat checked, which was reflected in the form of two waves (in the first and second half-years of 1919). Specifically, these two waves (they may be considered as one) produced the highest mortality rate, not the first wave which occurred at the beginning of 1918. Indirect evidence of the fact that the first wave was associated with the old virus, while the second and third waves were associated with the new one, is constituted by two facts. The first of them is the high mortality rate beginning with the autumn of 1918 (beginning of the second wave). The second fact is the appearance of epizootics of swine influenza also in the autumn of 1918 rather than earlier. Therefore, the influenza pandemic of

1918/19 in general spread in the same way as the last pandemic.

Its catastrophic nature (in contrast to the influenza A-prime pandemic) is apparently explained by the different socio-economic conditions of the time associated with the nature of the First World War, which caused a decrease in the specific immunity by virtue of the prolonged separation of countries, whereas during the influenza A-prime pandemics the immunity, so to speak, followed on the heels of the virus variations.

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EIGHTH CHAPTER

DIAGNOSIS

77. Clinical-Laboratory Methods of Diagnosing Influenza

The diagnosis of influenza, which does not offer any particular difficulties during epidemic outbreaks, is difficult during interepidemic periods. This to a considerable degree explains the high percentage of erroneous diagnosis which are encountered in the influenza hospital, which by far exceed the number of diagnostic errors in any other disease. The disease is readily confused with many infectious diseases, the onset of which is associated with catarrhal signs (measles, scarlet fever, typhus, typhoid and paratyphoid diseases, etc.). Influenza is frequently confused with acute diseases and exacerbations of chronic diseases of the respiratory tract, particularly of the upper respiratory tract. It can be confused with any disease in persons suffering from chronic pathology of the respiratory organs. All kinds of diseases accompanied by a general toxic state are taken for influenza.

In making the diagnosis use should be made of comprehensive clinical, epidemiological and laboratory data.

At the present time, various methods of laboratory diagnosis of influenza have been worked out; many of them have been recommended by the Ministry of Health USSR in the form of instructions in 1943 and have also been included in text books on virology (Shubladze and Gaydamovich, 1954) and laboratory diagnosis (Smorodintsev, 1949).

The principal methods of laboratory diagnosis of influenza used at the present time may be divided into two groups.

I. <u>Early Laboratory Diagnosis</u>
   a) Isolation of the virus.
   b) Hemagglutination reaction with nose and throat washings.
   c) Rhinocytoscopy.
   d) The complement-fixation reaction with the

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antigen from the patient's blood.

II. Retrospective Laboratory Diagnosis
   a) Detection of influenza antibodies in the blood of those who have had influenza.
   b) Laboratory diagnosis in pathological investigations.

The methods of investigation may be classified as clinical-laboratory, virology and serological. It goes without saying that this division is to a certain extent arbitrary, but it is convenient for purposes of description.

Clinical-laboratory methods of investigation in influenza, as in other infections, are not specific diagnostic methods; however, when used properly they can be of great assistance in the diagnosis of influenza. The most important methods of clinical-laboratory investigations are rhinocytoscopy and hematological examination (white blood analysis), along with which other methods of investigation are used.

Rhinocytoscopy. This clinical-laboratory cytologic method was worked out by Kolyaditskaya (1947, 1948) in the infectious disease hospital of the All-Union Institute of Experimental Medicine and was checked by her during two outbreaks (influenza A in 1946 and influenza B in December 1946-January 1947) as well as during the interepidemic period. The method has completely justified itself. Afterwards, the work was continued by Dubnyakova (1954). The method was checked by her and by other authors (Orlov and Kantsler, 1950; Fedorov, 1951; Undrits and Koldobskiy, 1953; Fotin, 1954, and others).

It consists of the investigation of cellular elements obtained from the nasal mucosa by means of impressions. Impressions are taken from the inferior nasal concha of the influenza patient on a small piece of glass about 10 centimeters in length, five to seven millimeters in width and two to 2½ millimeters in thickness which has been well ground in order not to injure the nasal mucosa. In case influenza is present, cells of columnar epithelium, usually degenerated and not uncommonly in entire layers, may be found in the impression.

The technique of obtaining the impressions is the following. The small strips of glass should be washed out well, defatted and kept in packages wrapped in paper.

Before introducing the strip of glass into the nose the patient's last name should be written on one side of it with a wax pencil. The other half of the strip of glass should be kept clean for the purposes of taking the impression.

The impressions may be obtained by two methods.

In the first method a head mirror and nasal speculum are used. The strip of glass is introduced cautiously into the nostril, applied to the mucous membrane of the anterior segment of the inferior nasal concha (but not to the nasal septum!) and, the strip of glass is carefully taken out, trying not to smear the impression. The glass strip is applied to the mucous membrane with the side on which the patient's last name has been written.

In the case of narrow nostrils, impressions may be obtained also from the mucosa of the nasal septum on the opposite side of the glass strip, and these should be rubbed off. Therefore, it is absolutely necessary to write the patient's last name on the glass strip first in order not to rub off the required impression from the concha. It is recommended that the impressions be obtained from both nostrils on separate glass strips.

The second method of obtaining the impression is without a head mirror or nasal speculum. After raising the patient's head and tip of the nose somewhat and after making sure that there is no marked deviation of the nasal septum, the glass strip is slowly and carefully introduced along the nasal septum as far as it will go. Then the glass strip is applied to the side opposite the septum and taken out along the nasal septum. The side which has not been marked with a waxed pencil should be rubbed off. The impressions are taken from both sides of the nose with different strips of glass.

If there is considerable mucous or purulent discharge on the strip of glass, and where the material is taken by means of the head mirror and nasal speculum, the mucus or pus may be removed carefully with a cotton tampon and the impression made after that. With the second method of taking the material, when there is considerable discharge present, it is recommended that two impressions be taken from each side of the nose. Then, there will be more mucus and pus cells on the first impressions, and epithelial elements on the second impression (if they have not sloughed off). In the second method of obtaining the impressions it is recommended that the patient be questioned about the existence of any nasal diseases and that the nasal cavity be examined. If there is a deviation of the nasal septum or a visible polypoid proliferation the impressions should be taken from one side, the one in which it is more convenient to reach the inferior concha.

The first method gives a higher percentage of positive results (up to 85 percent) than the second (up to 65 percent). Taking into consideration a certain degree of complexity of the method with the use of rhinoscopy, the

second, simplified method may also be used.

The impressions should be obtained without any effort or pressure, because this leads to trauma of the mucous membrane and incorrect results. A considerable number of red blood cells in the impression indicates bleeding or that the impression was taken incorrectly, and such impressions cannot serve as material for investigation.

Let us describe the method of staining. The dried impressions are placed on "rails" (glass or rubber tubes) over a tray with the smear up, and undiluted May-Grunwald solution, which serves as the fixative, is poured onto them. After a minute, and before pouring off the stain, an equal quantity of distilled water is added to the stain and mixed by rapidly taking up and blowing out the fluid from a pipette or blowing a stream of air from the pipette. This diluted stain is kept on for another minute. Then, the stain is poured off and the glass is placed vertically on filter paper so that the stain residuals drip off. Without using water irrigation, a solution of Romanowsky Giemsa stain is poured on in a concentration such as is effective in staining blood smears in the given laboratory (that is, one or two or three drops of the stain per cubic centimeter of distilled water). This stain is prepared every time directly before taking the impressions and is kept on the glass strip for no more than two or three minutes. A reduction in the time used for this stage of staining (from 10-15 to two or three minutes) has been introduced in our hospital by Dubnyakova. After this, the impressions are washed with distilled water and dried in the air (they should not be dried with filter paper).

The third stage of the staining may be modified, that is, instead of the Romanowsky-Giemsa stain solution the staining solution (azure-eosin) is poured on for three or four minutes, using the Nocht method.

The method of staining described is the best one, because it shows the cell structure and various changes in them, etc. well. However, for practical purposes where the problem is only one of the existence of columnar epithelial cells in the impressions, use may be made of simpler stains also. A simplified stain is, in principle, a stain which is accepted for blood smears in the given laboratory but with a shorter staining period. The impressions prepared by various methods are fixed after drying in air, using the same liquid fixative as is used for blood smears (redistilled ethyl or methyl alcohol, denatured alcohol, mixture of alcohol with ether) for two or three minutes. After drying the impressions are stained for 10-15 minutes with the stain used for blood (Roman-

owsky Geimsa or azure-eosin), washed with water and dried in air. It is not recommended that they be fixed over a flame.

Recently, in connection with works on the detection of specific inclusion bodies in cells of columnar epithelium of the nasal mucosa (Pigarevskiy and Chalkina, 1957) Dubnyakov in our hospital has used the staining of the impressions by the method of Koldobskiy with a certain change in the periods of staining. The impressions from the mucosa of the nasal conchae are dried in the air, not fixed, but rather immediately placed (vertically) in a narrow glass vessel containing undiluted May-Grunwald stain for four or five minutes, after which they are transferred to undiluted Romanowsky-Geimsa stain for four or five minutes also. After this, the impressions are rinsed successively in two glass containers with distilled water and are dried in air in a vertical position.

The basis for the use of rhinocytoscopy as a diagnostic method for influenza is the characteristic picture of the pathological processes in influenza, which is distinguished from acute catarrhs of the respiratory tract, which are similar to influenza in their clinical course.

The most important characteristic of the histologic changes in the mucous membranes of the upper respiratory tract in influenza are necrosis and desquamation of the columnar epithelium. In Fig. 226 a section of nasal mucosa of a practically healthy person is shown, which gives us an idea of its structure. The characteristic structure of the columnar ciliated epithelium attracts attention. In influenza the most pathognomonic feature is the early necrosis and desquamation of the columnar epithelium (Figs. 227, 228).

In contrast to influenza, in acute catarrh the columnar epithelium is not damaged (Fig. 229). These mucosal characteristics in healthy persons as well as in influenza patients and those with acute catarrhs produce different pictures in the impressions.

The impressions of the nasal mucosa of healthy persons are poor in cellular elements, and in places solitary columnar ciliated epithelium and leucocytes are encountered. There are more cellular elements in the morning, immediately after sleeping, than during the daytime. In influenza, squamous epithelium, leucocytes and sometimes histiocytes may be seen in the impressions in addition to columnar epithelial cells. The columnar epithelial cells are arranged in isolated fashion or in groups. Almost always the columnar epithelial cells are without cilia and are altered in a degenerative manner: the cytoplasm is vacuolated; the cell outlines are hazy;

the cells are reduced or increased in size, and frequently have only cytoplasmic residuals. The nucleus is pycnotic, and stains very well. The columnar epithelial cells are distinguished from the squamous cells, aside from their shape, in the fact that the nucleus is always closer to one pole. Columnar epithelial cells in the deeper layers are encountered which do not have a cylindrical but rather a cuboidal form.

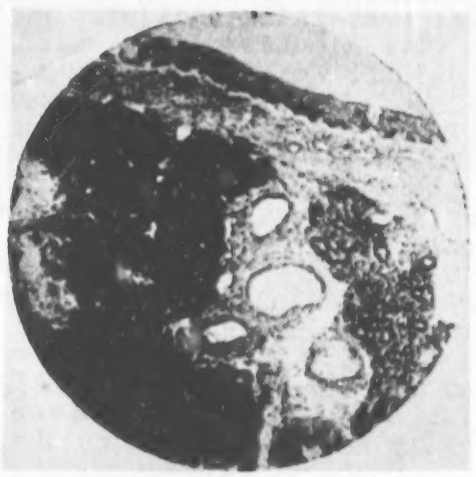

Fig. 226. Nasal Mucosa of Healthy Person (Malomuzh, 1948).

The positive result is given in pluses, depending on the number of columnar epithelial cells (+, ++, +++). Accumulations or layers of columnar cells are of great importance. When solitary or rare layers of these cells are present in the preparation the result is noted as one plus. An abundance of these layers is noted, respectively, by two and three pluses. A negative result is shown where only solitary columnar epithelial cells or none at all is found in the entire preparation. A doubtful result, $\pm$, is noted in the presence of isolated cells in rare microscopic fields. The number of cells is not of decisive diagnostic importance, because influenza may be accompanied by abundant (usually) or sparse (less often) sloughing of columnar epithelial cells. The nearer to the beginning of the disease the impression is taken the more frequently the abundance of columnar epithelial cells is found. In any impression it is necessary to note the day of the dis-

ease, because the great majority of positive results is obtained on the first or second day of the disease.

Fig. 227. Nasal Mucosa on the Second Day of Influenza. Desquamation of Columnar Epithelium (Malomuzh, 1948).

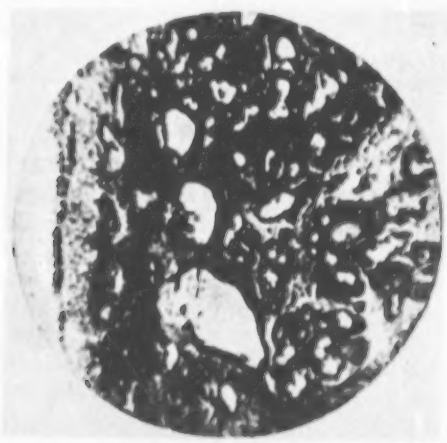

Fig. 228. Nasal Mucosa on the Fifth Day of Influenza. The Columnar Epithelium has Sloughed Off (Malomuzh, 1948).

Fig. 229. Nasal Mucosa on the Second Day of Acute Catarrh. Columnar Epithelium Not Damaged (Malomuzh, 1948).

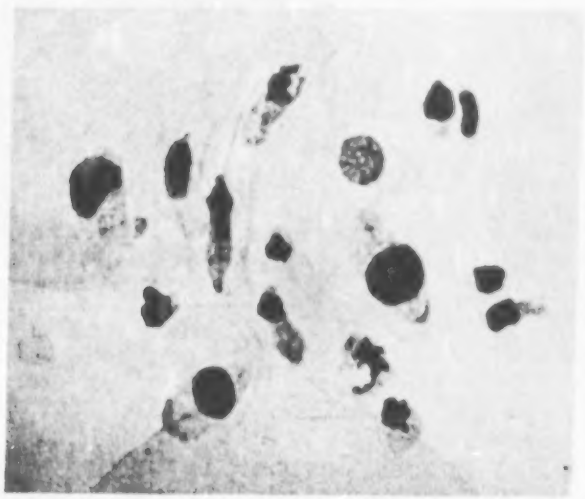

Fig. 230. Influenza. Ciliated Columnar Epithelium. Hypertrophy of the Cellular Elements (Kolyaditskaya, 1947).

We should like to present microphotographs of columnar epithelial cells from the work of Kolyaditskaya, who occupied herself with this method in the influenza department of the hospital (Figs. 230 and 231). These cells are usually degenerated. The cloudy swelling of the cytoplasm and the pycnotic nucleus are seen.

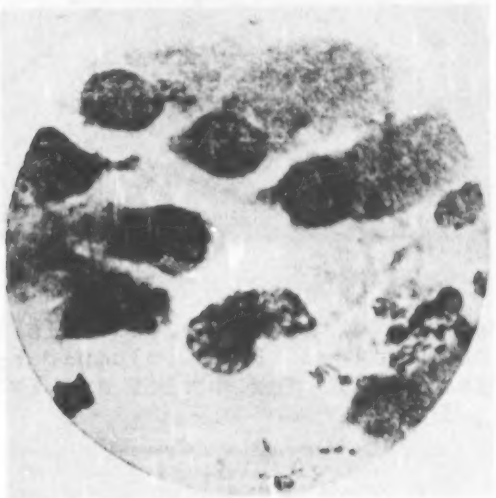

Fig. 231. Influenza. Ciliated Columnar Epithelium. Hypertrophy and Degenerated Cells (Kolyaditskaya, 1947).

Microphotographs are also shown of impressions taken from the nasal mucosa by Dubnyakova (Figs. 232 and 233). On them columnar epithelial cells are seen in which degenerated, vacuolated cytoplasm of the cells and pycnotic nuclei are noted.

In Fig. 234 two cells are shown which lie side by side, and on another microphotograph (Fig. 235) there is an entire layer of columnar epithelial cells.

Beginning with the first day of the disease a fair number of columnar epithelial cells may be observed in the impressions, and sometimes whole layers. The sooner the impressions are made the better the results which may be obtained. In the event of acute catarrh of the respiratory tract many pus cells are found in the impressions.

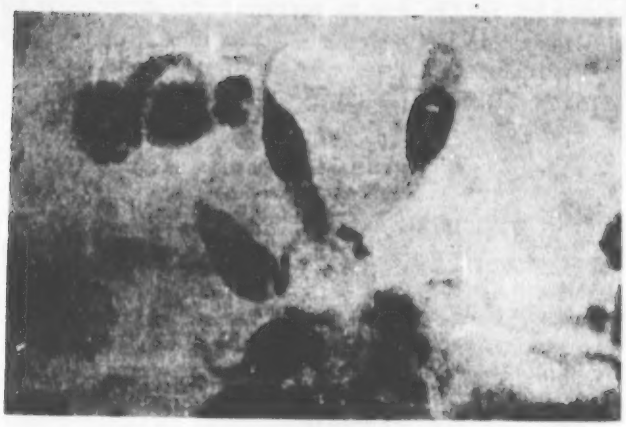

Fig. 232. Impression of Nasal Mucosa. Influenza. Second Day of Disease. Five Columnar Epithelial Cells are Isolated. The Nuclei are Pycnotic (Dubnyakova, 1954).

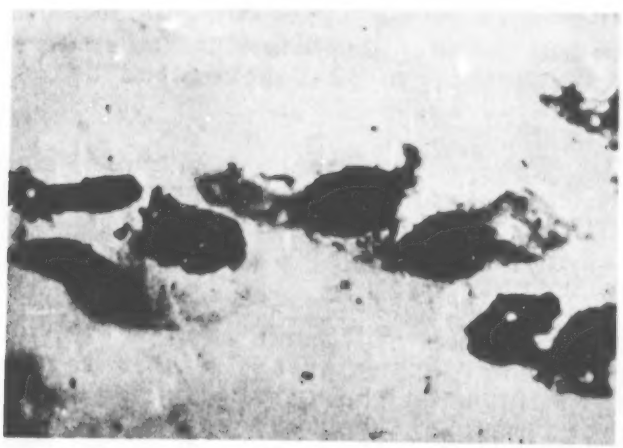

Fig. 233. Impression of Nasal Mucosa. Influenza. Group of Columnar Epithelial Cells with Vacuolated Cytoplasm (Dubnyakova, 1954).

Fig. 234. Impression of Nasal Mucosa. Influenza. Two Tremendous Swollen Columnar Epithelial Cells. Vacuolization of the Cytoplasm, Indistinct Nuclear Contours. In the Lower Cell There are Residuals of Cilia (Dubnyakova, 1954).

We should like to present also a microphotograph from the work of Kolyaditskaya (Fig. 236). In this impression there are many pus cells which are observed in acute catarrh. The columnar epithelial cells may be encountered in the form of rare solitary specimens.

The more time which has elapsed after the onset of a case of influenza the fewer the columnar epithelial cells in the impressions and the more pus cells there are. In acute catarrh of the respiratory tract there is a fair number of pus cells from the very beginning of the disease present in the impressions, and the number of columnar epithelial cells increases with the increase in time which elapses from the onset of the disease. Such a dynamic examination of the impressions shows the differential diagnostic features between influenza and acute catarrh of the respiratory tract.

A correction should be made with respect to chronic nasal diseases. In chronic nasal diseases, for example, polyps or chronic antral sinusitis columnar epithelial cells may be found in the nasal mucosa impressions even in the absence of influenza. This circumstance should be

taken into account in making the diagnosis. However, in such cases there are no changes in the condition of the columnar and pus cells in repeated examinations of the impressions.

Fig. 235. Impression of Nasal Mucosa. Influenza. Group of Cells (layers) of Columnar Epithelium. In the Majority of Cells the Cytoplasm Has been Destroyed. Vacuolization of the Majority of the Nuclei (Dubnyakova, 1954).

A similar picture (presence of columnar epithelial cells) may be observed in influenza in impressions taken from the nasopharyngeal mucosa (pharyngocytoscopy). This has been shown in the patients of our hospital by Fotin (1954, 1955), the house-surgeon of the ENT Hospital of the Second Moscow Medical Institute (director of the hospital --Professor B. S. Preobrazhenskiy). The impressions were made by means of a simple instrument which he proposed, which was introduced into the nasopharynx through the mouth.

A special glass strip is attached to the instrument, and on it an impression is taken from the pharyngeal surface of the soft palate by applying the instrument with the glass strip to it. The technique is very simple and can be carried out by any physician. The impressions are obtained in a purer form than those from the nose. We are presenting a microphotograph of an impression taken from

the soft palate of an influenza patient (Fig. 237).

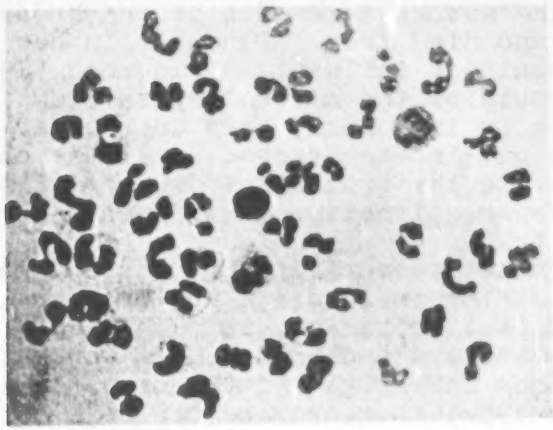

Fig. 236. Acute Catarrh of the Respiratory Tract. Neutrophils in Nasal Discharge (Kolyaditskaya, 1947).

Fig. 237. Pharyngocytoscopy; Impression From Posterior Wall of the Soft Palate. a--Columnar Epithelial Cells; b--Nuclei of Degenerating Cells; c--Leucocytes (Fotin, 1954).

It is interesting to compare the presence of desquamated columnar epithelium in the bronchi with the rhinocytoscopic and pharyngocytoscopic pictures in influenza. This has been observed by Afanas'yeva (1952) in the Model Children's Hospital in Moscow by obtaining impressions from the mucous membranes of the bronchi in autopsies of children who died from influenza; in her opinion this is pathognomonic of influenza. Zadorova (1953) has pointed out the necrosis of the mucosal epithelium in the accessory nasal sinuses in influenza; Hers and Mulder (1951) have pointed this out in the trachea. All the observations mentioned attest to the process of degeneration and desquamation of columnar epithelium in influenza throughout the respiratory tract.

The rhinocytoscopic investigations mentioned were carried out during influenza A and B outbreaks with the same results (Kolyaditskaya, 1948).

Kolyaditskaya's data have, in general, been confirmed by other investigators (Fedorov, 1951; Nenikova-Bogdanovich, 1952; Khomenko and others, 1952).

Krivaya-Usherenko (1952) modified the examination method somewhat by using nasal irrigation with physiological solution with a subsequent count of the leucocytes which have emigrated instead of taking impressions. The results which she obtained have been confirmed by Kolyaditskaya's data.

Hematology. In almost all cases of influenza, with the exception of complicated cases, there is a leukopenia beginning with the third day of the disease. On the first day, it is observed in approximately 25 percent of the patients, increasing to 75 percent by the third day of the disease. The absence of eosinophils, a decreased number of eosinophils, neutropenia, relative lymphocytosis (an absolute lymphocytopenia may be observed), monocytosis, toxic granulation of the neutrophils, thrombocytopenia and usually a normal sedimentation rate are observed. In acute catarrhs a normal or slightly increased leucocyte count may be noted. No neutropenia, toxic granulation of the neutrophils, absence of eosinophils or lymphocytosis or monocytosis is noted; the sedimentation rate is frequently more or less accelerated. This is apparently associated with the chronic processes in the respiratory organs, pharynx, ears, accessory nasal sinuses frequently observed in acute catarrhs as the result of the effect of the microbes living there.

Color Precipitation Reaction of the Urine. Yudkevich (1952) has performed a color precipitation test of the urine in patients with influenza and acute catarrhs of the respiratory tract. This was worked out in the

Soviet Union by Kimbarovskiy (1950), and is observed by the addition of silver nitrate to the urine (darkening of the urinary precipitate from hardly noticeable to marked). She found a positive and a markedly positive test in influenza in 95 percent of the cases, and in acute catarrhs of the respiratory tract, in 63 percent of the cases during the first few days of the disease.

According to the observations of Dubnyakova, this method was unsuitable for the differential diagnosis, because the precipitate is obtained in various infections and intoxications.

She examined more than 400 influenza patients and patients with acute catarrhs and did not find any essential difference in the frequency of occurrence of the precipitate in them (93 percent in influenza; 86 percent in catarrhs). Therefore, this reaction cannot serve as a basis for the differential diagnosis of influenza.

78. Virological Methods.

Among the virological methods of influenza diagnosis the following have been applied:

1. Isolation of the influenza virus in chick embryos, laboratory animals (ferrets, mice, rats, and others) and tissue cultures.
2. Virological examinations which have the aim of detecting elementary bodies and inclusion bodies.
3. Detection of the virus in the investigated material by means of the hemagglutination reaction.
4. Detection of the virus in investigated material by means of immunologic reactions--agglutination of elementary bodies, complement-fixation reaction, precipitation, agglutination of bacteria or collodion particles loaded with the virus.
5. Indirect methods of detecting the influenza virus by means of investigating the electrokinetic potential of the red blood cells and the condition of the receptors in the nasopharynx.

Methods of Isolating the Influenza Virus. At the present time, the method of isolating influenza viruses in chick embryos by means of infecting them with the investigated material in the amniotic or allantoic cavity (the former method is preferable because of its greater sensitivity) has become most widespread at the present time, and so to speak, constitutes a monopoly.

Zakstel'skaya (1954) used the interesting modification of isolating viruses in laboratory animals, and this may find practical application. As is well known, the main defect of the method of isolating influenza viruses

in mice is the low degree of sensitivity of this method, depending on the fact that the influenza viruses produce a definite clinical picture in mice (fatal pneumonia with accumulation of hemagglutinin in the lung tissue) only after adaptation. In this process, by far not every strain (particularly of B virus) becomes pathogenic for mice in the process of serial passages. At the same time, the non-pathogenic (unadapted) strains usually multiply well in the mouse lungs, accumulating in tremendous quantities and producing an immune reaction. This circumstance has also been taken in consideration by Zakstel'skaya. She investigated nasopharyngeal washings of persons immunized with influenza vaccine, and infected mice intranasally with these washings. She concluded that the virus was present in the washings because of the appearance of antibodies in mice the blood of which had been examined for the presence of antibodies two or three weeks after the infection. In making use of this method (immune mirror), Zalstel'skaya not only was able to establish the presence of vaccine virus in the nasopharynx of those innoculated but also to show the presence of three viruses (A, A-prime and B) in the nasopharynx of people innoculated with live polyvalent vaccine. Therefore, there is a point in checking this method of investigation more extensively for the purpose of establishing the etiology of influenzal diseases, particularly since it is economical: one or two mice are required for investigating the washing.

Among the methods of early diagnosis of influenza studied is viroscopy according to the Morozov method. Morozov (1951) showed that if a smear is made from the nasal mucosa of a patient with influenza (for example, with a probe covered with cotton), spread out on a glass slide and stained by the silvering method, the elementary bodies of the influenza virus may be detected by light microscopy. Merkulova, Kazanskiy and Khomazyuk (1954) have given a good report about this method in influenza.

In Figs. 238 and 239 microphotographs are presented of smear preparations taken from the conjunctivae of people sick with influenza which have been given to us through the kindness of Morozov and Peregudova.

In the influenza hospital Knyazeva has carried out investigations of influenza patients, utilizing smears taken from the nasal mucosa, and has shown structures similar to the elementary bodies of influenza virus described by Morozov (Figs. 240 and 241). The same structures were found in the nasal cavity of persons vaccinated by the introduction of live tissue vaccine into the respiratory tract (Fig. 242). Despite the fact that these structures have not been found to date in the nasopharynx of healthy

persons, we are still refraining from any conclusions as to their specificity.

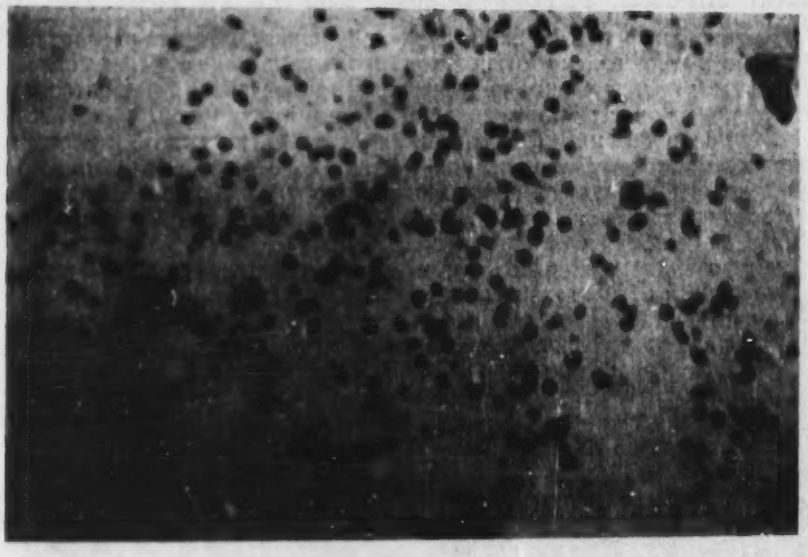

Fig. 238. Elementary Bodies of Influenza Virus (February 1953). Smear From Human Conjunctiva. Silvering According to the Morozov Method. Magnification 4,000 (Morozov).

We are presenting the method of silver staining by the Morozov method, according to a description given in the work of Merkulova, Kazanskiy and Khomazyuk (1954).

A fine smear is prepared on an ordinary glass slide. The excretions from the nasal cavity are taken from the inferior concha with a cotton tampon.

Three solutions are prepared for silver staining by the Morozov method: No 1 (Ruge's solution), which consists of one cubic centimeter of glacial acetic acid plus two cubic centimeters of 40 percent formalin and 100 cubic centimeters of distilled water; solution No 2 consists of five grams of tannin and one cubic centimeter of fused phenol plus 100 cubic centimeters of distilled water; No 3 solution--silver nitrate (five grams in 100 cubic centimeters of distilled water). Twenty cubic centimeters are poured out, and to the remaining 80 cubic centimeters a strong ammonia solution is added drop by drop until a yel-

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lowish-brown color appears and until a dark brown sediment appears, which is then dissolved by the remaining 20 cubic centimeters of silver solution until the appearance of a pale opalescence. The solution is freed of precipitate by filtration through filter paper, and it is kept for future use. For staining purposes, this solution in distilled water 1:10 is used.

Fig. 239. Elementary Bodies of Influenza Virus (February 1954). Smear From Human Conjunctiva. Silvering According to the Morozov Method. Magnification 3,000 (Peregudova).

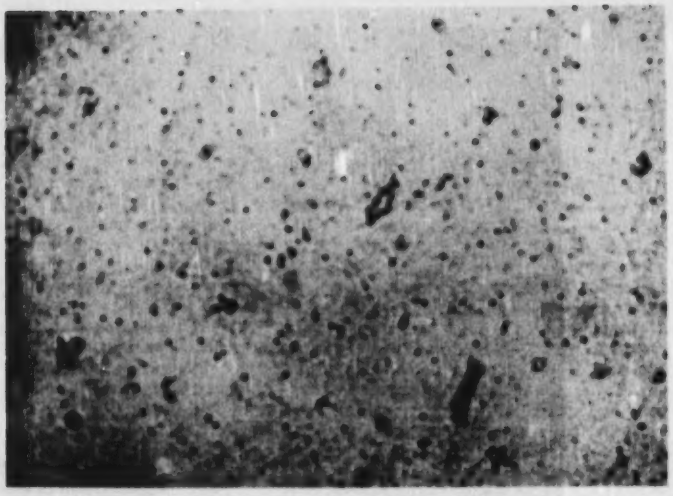

Fig. 240. Viroscopy. Smear From Nasal Mucosa. Patient M. Influenza A. Four-fold Rise in Antibody Titer. Preparation by L. D. Knyazeva.

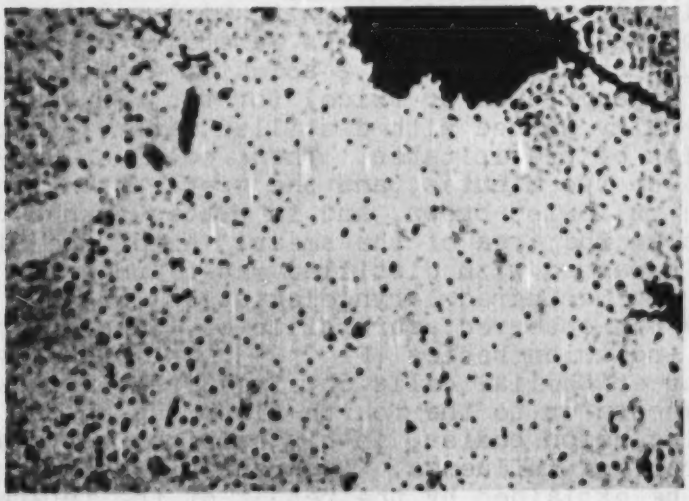

Fig. 241. Viroscopy. Smear From Nasal Mucosa. Patient K. Influenza A-prime. Second Day of the Disease. Sixteen-fold Rise in Antibody Titer. Preparation by L. D. Knyazeva.

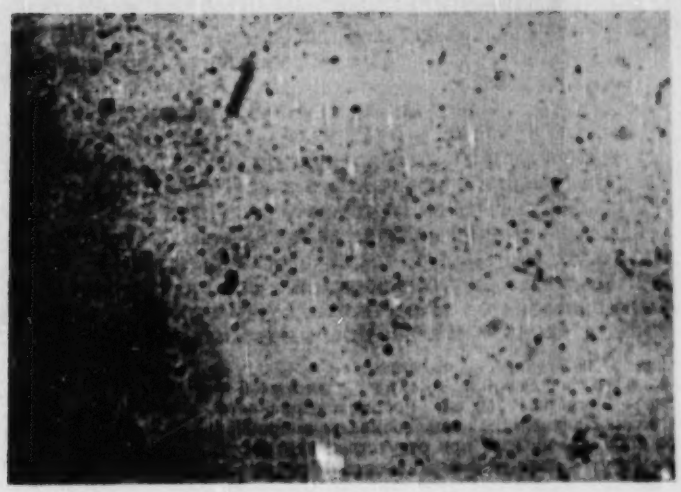

Fig. 242. Viroscopy. Smear From Nasal Mucosa. Person Vaccinated With Bets. Influenza Vaccine (Type B). Preparation by L. D. Knyazeva.

Staining of the Smears. The smear which has been dried in air is poured over with No 1 solution (Ruge's) for one minute; then, this is carefully washed off with water and treated with No 2 solution with heating until vapors appear (once or twice) for two minutes. The smear is carefully washed with distilled water to avoid the formation of precipitates. Then, solution No 3 is applied for one or two minutes, and the preparation is heated until a yellowish-brown, and in places even a black color, appears in the area of the smear. After the silvering the smear is washed with distilled water and dried in air.

The Leningrad laryngologist Koldobskiy, points out that he has observed azurophilic or (less often) basophilic inclusion bodies (Fig. 243) in influenza in the columnar epithelial cells obtained from impressions of the nasal mucosa up to the fourth-sixth day of the disease. These inclusion bodies, in his opinion, serve as a reliable proof of influenza. Apparently, their occurrence should be explained by the result of the influenza virus effect on the susceptible cell (Undrits and Koldobskiy, 1953).

Similar inclusion bodies (basophilic and acidophilic) have been observed by Pigarevskiy (1953) in the epithelium

of the bronchi and trachea of mice infected with influenza virus, and later (1957), in persons sick with influenza.

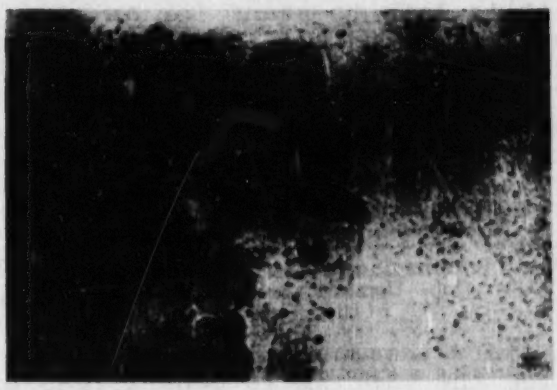

Fig. 243. Inclusion Bodies of Multiplying Influenza Virus in Cytoplasm of Columnar Epithelium of Nasal Mucosa (Undrits and Koldobskiy, 1953).

The hemagglutination reaction consists in the detection of influenzal hemagglutinins in the contents of the upper respiratory tract. It is based on the capacity of the influenza virus of being adsorbed onto the surface of erythrocytes with subsequent agglutination of them, which is readily distinguishable with the naked eye (Hirst, 1941; McClelland and Hare, 1941). The reaction was introduced into the practice of laboratory diagnosis of influenza by Soviet investigators (Solov'yev, Shubladze and Mel'tser, 1943; Smorodintsev and Drobyshevskaya, 1943; Peterson and Semashko, 1945).

In evaluating the results of the practical significance of the hemagglutination reaction it should be taken into consideration that its specificity depends on the use of accurately titrated and carefully prepared influenzal antisera. Agglutination of the erythrocytes without a check with specific sera does not have any diagnostic importance. The latter is based on data indicating the presence of nonspecific hemagglutinins in the saliva of healthy people. It should also be kept in mind that not only the virus of influenza but also certain other viruses and bacteria are capable of causing erythrocyte agglutination. Specifically, in this group are the virus of mumps and the pathogen of pertussis (H. pertussis).

The Hemagglutination-Reaction Method. The patients gargle (no less than one minute) with 10 cubic centimeters of sterilized saline solution. The washing obtained is collected in a test-tube, centrifuged for five minutes at 2,000 revolutions per minute, or, if there is no centrifuge available, it is left to stand for an hour. The supernatant is used for the reaction. In young children sterile cotton tampons previously moistened with three cubic centimeters of hormonal bouillon are used for the purpose of obtaining material. The throat and the nasal cavity are rubbed separately, and the mucous obtained is rinsed out into the bouillon indicated above, after which a second wiping of the nose and throat is accomplished with the aim of increasing the quantity of test material using the same tampons. The material obtained by this method is placed into a small cup with beads (glass). The tampons, which are carefully removed from the sticks with a sterile forceps are put into the same place. After a vigorous three-minute shaking the washing is centrifuged by the method indicated above and used for the test.

For the purpose of taking material from small children, Speranskiy (1950) recommends the use of a fine, so-called "ureteral" catheter. The catheter is attached to a one-two gram syringe in which one to 1½ cubic centimeters of physiological solution is preliminarily taken up, passed directly to the posterior wall of the pharynx, and the contents are taken from there. In this way, it is possible to take material for the hemagglutination reaction or for the purpose of isolating the virus in the youngest children.

Guinea-pig erythrocytes are used most frequently as the indicator of the influenzal hemagglutinins. For this purpose use may be made of chick erythrocytes as well as human erythrocytes with group O blood. The erythrocyte suspension is prepared from defibrinated blood, which is obtained either by shaking it in a jar with glass beads, or by the addition of five-percent sodium citrate solution. In the latter case, one part of this solution is added to two parts of freshly-taken blood. The defibrinated blood is subjected to repeated centrifugation and washing with physiological solution three or four times. After the extraction of the supernatant fluid, which should be almost colorless, the necessary cencentration of washed erythrocytes is prepared from the precipitate.

Five-tenths of a cubic centimeter of the washing is taken for the reaction and it is combined with 0.5 cubic centimeter of 0.5 percent erythrocyte suspension. Test-tubes containing the mixture of the washing and the erythrocytes are carefully shaken up and left at room temperature for one or two hours. For the purpose of control,

0.5 cubic centimeter of the physiological solution is added to the erythrocyte suspension. The result of the reaction is examined twice: after 20 minutes and after one hour. When the test is positive the agglutinated erythrocytes precipitate to the bottom of the test-tubes in the form of a friable precipitate with uneven umbellate edges. In the absence of agglutination (negative result) the erythrocyte precipitate on the bottom of the test-tube is compact, with evenly outlined edges.

The specificity of the agglutination is determined by the neutralization (inhibition) of it induced by the addition of influenza immune antisera of types A and B. For the given experiment a preliminary titration of hemagglutinins of the washing is recommended, determining the final dilution of it (using two-fold dilutions) which is capable of agglutinating erythrocytes. This final dilution of the washing is arbitrarily designated the hemagglutinating unit. In the experiment each washing is poured out in a quantity of 0.25 cubic centimeter into three series with equal numbers of test-tubes in dilutions containing different numbers of hemagglutinating units in an ascending order. Influenza antiserum type A is added to the first series; influenza antiserum type B, to the second series; normal non-immune serum (control) is added to the third series. Then, 0.5 cubic centimeter of 0.5-percent erythrocyte suspension is introduced into all the test-tubes. After shaking the test-tubes the mixture is left at room temperature, and the results of the reaction are determined after twenty minutes and after one hour. If the agglutinations of the erythrocytes are specific it becomes negative under the influence of one of the immune sera. This occurs because of neutralization of the virus with influenzal antibodies--antihemagglutinins, which type the hemagglutinins of the washing and indicate the specificity of the reaction.

The sensitivity of the hemagglutination reaction can be increased by means of increasing the volumes of tested washings. In the given modification of the test (according to Smorodintsev) a washing obtained by means of five rinsings of the throat with physiological solution, with 10 cubic centimeters for each rinsing, serves as the test material. The washing is collected in a volume of 50 cubic centimeters in a wide-necked jar with a ground stopper or in a flask of the appropriate size. The collected material is quickly delivered to the laboratory, where the washing is clarified by centrifugation (30 minutes at 2500 revolutions per minute) or by filtration through a compact cotton filter. The clarified washing is poured out into three test-tubes having a diameter of

1.75-2.5 centimeters using 10 cubic centimeters in each. Type A (first test-tube) influenza antiserum, type B (second test-tube) antiserum and normal serum (third test-tube-control) are poured into the test-tubes. Seventy-five hundredths of a cubic centimeter of one-percent suspension of washed erythrocytes are put into all the test-tubes. The test-tubes containing the mixture are shaken up and left at room temperature for three or four hours or are left for the night in a refrigerator at a temperature of two to 10°. The erythrocytes are kept at a temperature of four or five degrees for no more than four days, in the washed-out form for the hemagglutination reaction. The diagnostic influenza antisera are used in dilutions which correspond to a titer of no less than 1:20.

We have presented here the simplest and the most frequently used method of performing the hemagglutination reaction with a washing taken from the nasopharynx. A more detailed review of these methods has been presented in the second chapter; there the characteristic features of this reaction and the nature of it were indicated.

After the publication of the first few works on the use of the hemagglutination reaction with washings this method of early diagnosis of influenza began to be incorporated extensively into practice. Investigations carried out in the 1940's made it possible to conclude that this reaction quite accurately reflects the influenza morbidity rate among the population when performed in large scale investigations (Maksianovich and others, 1948; Reznikova and Marisova, 1950).

In Lukshira's work (1950) carried out in conjunction with Zhdanov, a very distinct relationship was observed between the frequency of positive hemagglutination reactions and the course of the influenza morbidity rate. The investigation was carried on from October 1948 through May 1949. During this time there were two waves of influenza: a small seasonal rise in December-January (influenza A) and a considerable outbreak in March-April (A-prime influenza, as was afterwards established). In complete accordance with the progress of the morbidity rate the percentage of washings producing a positive reaction increased to 17 in January, to 32.5 in April (Fig. 244); the increase in the number of the washings which produced a positive reaction occurred entirely in the group of the type A virus.

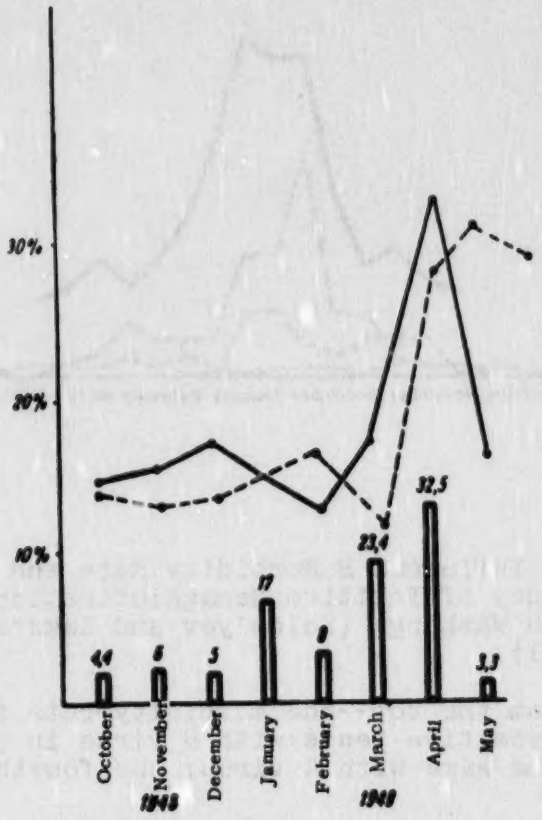

Fig. 244. Rise in Incidence of Virus Influenza A in March-April 1949 (Lushkina, 1950).

_____Total morbidity rate: --- percentage of antihemagglutinins; the columns represent the percentage of positive washings.

During the influenza B outbreak in 1950 Solov'yev and Zakstel'skaya (1951) observed a very similar picture (Fig. 245). The increase in the percentage of positive reactions coincided with the increase in the influenza morbidity rate, and the type of virus in the reactions cor-

respond to the etiology of the diseases during this epidemic.

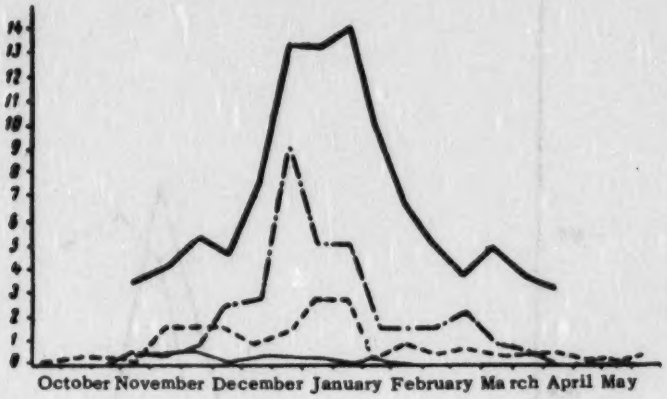

Fig. 245. The Influenza B Morbidity Rate and the Frequency of Positive Hemagglutination Reactions with Washings (Solov'yev and Zakstel'skaya, 1951).

First curve from the top--the morbidity rate for the city; second curve, positive tests with B virus in percent; third curve, the same with A virus; the fourth curve, positive AB tests.

Similar data have been presented by Barats (1952) in Ufa, Fedorov (1952) in Sverdlovsk, as well as by Shavrova and Klemina (1953) in Moscow.

It should be stated that the hemagglutination reaction in the Soviet Union has been used quite extensively, and much more so than abroad.

The expediency of incorporating it into practice as one of the methods aiding in making a differential diagnosis of influenza and acute catarrhs has been pointed out by Zhdanov (1952, 1953), Khomenko and co-authors (1952, 1953), Ritova (1953).

At the same time, the experience which has been accumulated in these years has shown the serious defects of this test: its inadequate sensitivity and low degree of specificity, making it unsuitable for individual diagnosis.

With rare exceptions the percentage of positive re-

actions in cases known to be influenza did not exceed 25-30 and usually was lower (Ritova, 1953). In connection with the low degree of sensitivity of this reaction suggestions were made to increase it by means of preliminary concentration of the antigen on erythrocytes (Peterson, 1948; Peterson and others, 1948; Sokolov, 1949). However, this modification has only complexified the method of examination without improving its results, as has been shown by the checking work of Lozhkina (1953).

The low degree of sensitivity of the reaction depends on two main causes: an inadequate quantity of the virus in the washing and the presence of inhibitors in the washing (Francis and Minuse, 1948), which mask the virus. The suggestions of Peterson and Sokolov had the aim of artificially increasing the virus concentration and, by the same token, increasing the sensitivity of the reaction; for the partial removal of inhibitors it was suggested that the washing be allowed to stand or be filtered, which did not always give the anticipated results.

Another defect in the hemagglutination reaction is its low degree of specificity--it is positive in healthy persons and in patients with other diseases besides influenza; here, various types of nonspecific reactions are observed. Usually, they are distinguished from the specific reactions by the fact that they are not inhibited by immune sera.

The nonspecific results of the hemagglutination reaction with washings depend on many causes. One of them is the presence of nonspecific hemagglutinins in the nasal secretion, the saliva (as well as in the sera) of healthy persons (Fridman, 1952, 1953). The main source of the nonspecific hemagglutinins is saliva; another source is the small mucous glands of the nasopharynx. This hemagglutinin is a colloid, passes through Rublev Nos 1 and 2 filters, is precipitated by centrifugation at 4,000 revolutions per minute for 30 minutes, withstands heating to 56° and is inactivated at 90-100° (it is probably a protein). Human erythrocytes are not very sensitive to this hemagglutinin, and guinea-pig erythrocytes are very sensitive (Kantorovich, 1954).

Another cause of nonspecific results in performing the hemagglutination reaction with washings is a depression of its serum by inhibitors (see second chapter). The methods of destroying mucoid inhibitors have now been worked out in detail (Tyrell and Horsfall, 1952; Kozinski and Val'kovskaya, 1952). It is to be desired that they be more hastily introduced into the production of diagnostic sera.

In connection with these hemagglutination reaction

defects in the method of early diagnosis of influenza Smorodintsev and co-authors (1952, 1953) in 1951 suggested replacing the guinea-pig erythrocytes, which were utilized in the laboratory, by human erythrocytes of blood group O in the following modifications:

a) Obtained washings from patients for eight to 12 hours by means of repeated rinsings of the throat with 10 cubic centimeters of physiological solution--for the purpose of taking 100-120 cubic centimeters of the full-value rinsing material;

b) Performing the hemagglutination reaction in 20-30 cubic centimeters of the washing rather than in two cubic centimeters of the washing as recommended by the instructions and using a minimum dose of 0.5 cubic centimeter of one-percent suspension of erythrocytes of human blood group O;

c) The use of polyvalent active specific A, A-prime and B sera for inhibiting hemagglutination.

Investigations in which the high degree of sensitivity of human erythrocytes was shown (Fig. 246) as well as the data of Kantorovich presented above concerning the sensitivity of guinea-pig erythrocytes to nonspecific hemagglutinins have served as the basis for replacing guinea-pig erythrocytes with human erythrocytes. According to the data of Shishkina and Yurikas (1953), increasing the volume of the washing increased the percentage of positive reactions from 14 to 23.4, and, according to the data of Fridman and Khayt (1953), increased the total of positive reactions for epidemic and interepidemic periods from 1.1 to 8.6. Similar data have been obtained previously by Kantorovich and co-authors (1951). The suggestion that guinea-pig erythrocytes be replaced by human erythrocytes was approved and recommended for practical use in the official instructions on the diagnosis of influenza (1951) and played an extremely negative part in the subsequent application of this reaction for practical purposes. Considerable work in incorporating the hemagglutination reaction for the diagnosis of influenza into the practical laboratories of Moscow made it possible to evaluate the practical unsuitability of the new modification of the test (Orlova and co-authors, 1952, 1953; Samvelova, 1953). Samvelova presents the following data concerning the results of testing of various modifications of the test under practical conditions. In 1949, the laboratories in Moscow used chick erythrocytes and positive reactions were obtained in 25-35 percent of the patients with influenza; in 1950/51--guinea-pig erythrocytes, and 10-15 percent positive reactions were obtained; in 1952--human blood group O erythrocytes, and the percentage

of positive reactions came down to almost nil (0.6-1.2). For the purpose of a proper evaluation of these data it is essential to keep in mind the fact that during three years more than 20,000 examinations were performed in the laboratories of the city, and the results of the examinations were compared with the movement of the influenza morbidity rate (Fig. 247). From the figure it is seen that while in 1949, 1950 and 1951 the frequency of positive hemagglutination reactions was a more or less satisfactory reflection of the movement of epidemic influenza, in 1952 the application of this reaction gave truly pitiful results.

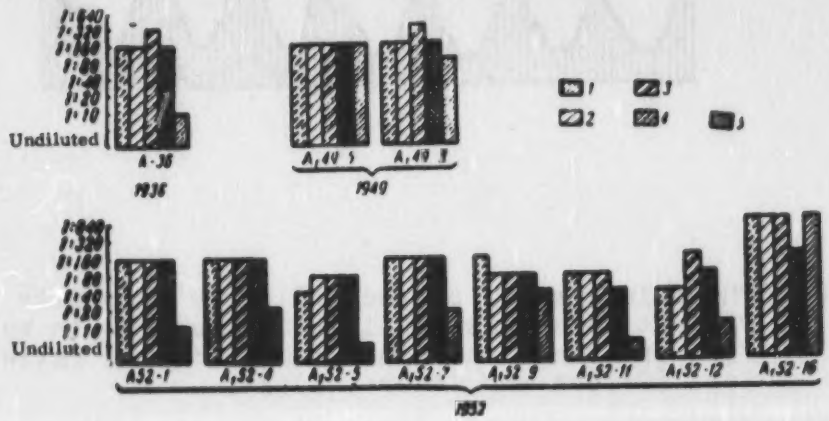

Fig. 246. Power of Influenza Virus of Agglutinating Erythrocytes of Different Species of Animals, Birds and Man at Zero Degrees.

1 -- chick erythrocytes; 2 -- guinea-pig erythrocytes; 3 -- human erythrocytes; 4 -- dog erythrocytes; 5 -- sheep erythrocytes (Smorodintsev and others, 1953).

According to the data of Khomenko and co-authors (1954), the new method is a failure not only because of its low degree of sensitivity but also because of a large number of nonspecific reactions.

It is not surprising that in recent works the authors themselves (Smorodintsev, Klyachko and others, 1953) rejected the method which they had suggested for performing the hemagglutination reaction, excluding it from the group of methods recommended for diagnosing influenza.

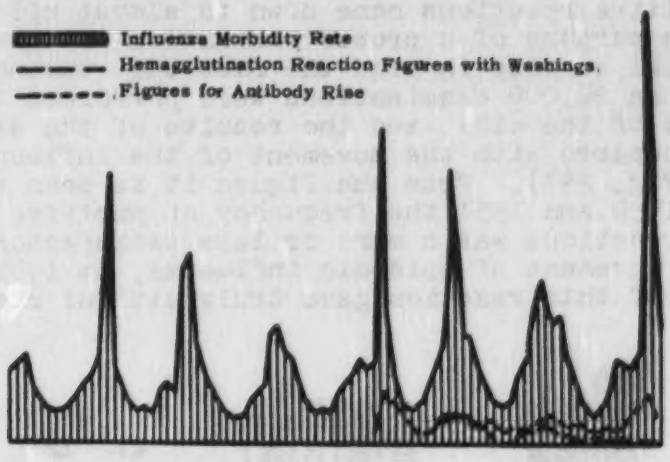

Fig. 247. The Relationship of the Influenza Morbidity Rate Figures, the Hemagglutination Reaction in Washings and the Rise in Antibody Titer in Patients Sera (Orlova and others, 1953).

Nevertheless, there are no grounds for rejecting the use of this reaction. The failures associated with the inadequately planned and gradual incorporation of the modification of this test do not necessarily vitiate the test itself, which is based on the use of one of the cardinal properties of the influenza virus for the diagnosis of hemagglutination. We, therefore, considered it necessary to do work in checking and improving the hemagglutination reaction (Zhdanov, 1954; Lozhkina, 1954). This was particularly essential since, according to the investigations of Solov'yev and Zakstel'skaya (1954) made in this laboratory the modifications previously used gave up to 25 percent positive results in comparison with the clinical diagnoses of influenza and 40 percent with respect to cases of serologically established influenza.

Lozhkina occupied herself with the checking and improvement of the hemagglutination reaction. An important condition for the obtaining of reliable results should be the confidence that the influenza virus is contained in the washing being investigated. Naturally this condition is difficult to fulfill even during the period of an influ-

enza outbreak, but particularly in the interepidemic period. Therefore, volunteers were used as models for the investigation who had been vaccinated with tissue strains of the influenza virus, and in them, because of the high survival rate of the strains and active multiplication of them in the nasopharynx, it was possible not only to determine the existence of the virus reliably but also to find out the quantity (virus titer) in one cubic centimeter of the washing.

The first part of the investigations made it possible to show quite distinctly that of the three types of erythrocytes -- guinea-pig, chick and human (blood group 0) -- the guinea-pig erythrocytes were most sensitive, while human erythrocytes were least sensitive and not very sensitive. As far as specificity is concerned, the percentage of nonspecific reactions from the use of the same method of performing the reaction was approximately the same for all three types of the erythrocytes. Therefore, the failures in using the hemagglutination reaction in the modification approved by the instructions were quite consistent.

The next group of investigations made it possible to show which volume of washing was optimal in performing the test. A comparative use of the reaction with volumes of 0.5, 5 and 20 cubic centimeters (latter modification had been recommended by Smorodintsev) showed that the volume in two cubic centimeters is optimal both in the number of positive results and in their specificity.

Smorodintsev's suggestion that washings be used which had been collected for six hours did not improve the results of the test. A two-fold concentration of the virus from washings onto erythrocytes did not improve the results of the reaction either. During the course of these investigations it was shown that filtration of the washings through cotton which was tightly packed into a syringe considerably reduces the number of nonspecific reactions.

Concentrating on this modification of the reaction (filtration of the washing through cotton, performance of the test in the volume of two cubic centimeters, and the use of guinea-pig erythrocytes), Lozhkina obtained a positive hemagglutination reaction in 30 percent of volunteers vaccinated with the A-prime virus.

This test was positive with the same degree of frequency in patients during an outbreak of influenza in the autumn of 1953. In comparing the results of the hemagglutination test with the virus titers in the nasopharynx it was shown that the test is more often positive with a high virus concentration in the washing (from 10^2-10^6 embryo-infecting units per cubic centimeter of the washing), whereas negative tests usually occur when the virus is present in

the washings in low concentrations (10^1 or 10^0).

The investigations made, therefore, show that the hemagglutination reaction with washings is a promising method for the early diagnosis of influenza but needs further perfection -- increase in the sensitivity and elimination of nonspecific reactions.

Among the methods of early laboratory diagnosis of influenza is also the complement-fixation reaction with antigen from the patient's blood. According to the material of Smorodintsev (1948), Zakstel'skaya (1949), Shishkina and Yurikas (1953), this reaction gives positive results in the diagnosis of influenza during the first few days of the disease. The serum of the patients is used as the antigen in it; sera of those who have recovered from influenza or immunized animals are used as the immune sera. The sensitivity of the reaction is increased when it is performed according to the Ioffe method (prolonged fixation at a temperature of 4-5°).

Subsequently, Zakstel'skaya (1952, 1953) successfully used the complement-fixation reaction for detecting the influenzal antigen in the urine of patients. The latter is more often found in children (in 6 of 17 investigated) than in adults (in 2 out of 18 investigated). A complete correlation of antigen findings in the urine with the antibody changes in the blood of the patients was noted.

The complement-fixation reaction for the detection of antigen in the nasopharynx of influenza patients began to be used practically after Smorodintsev (1952) worked out a new system of performing thetest based on an increase in the volume of the washing being investigated from 0.1 to 5 cubic centimeters (that is, by 50 times) while the usual volumes of all the other components was kept the same (0.2 cubic centimeter), whereby an increase in the sensitivity of the reaction is provided. We should like to present a description of it according to Smorodintsev and co-authors (1953).

A washing from the patient's throat is taken by the usual means or is collected for several hours by means of repeated garglings (the washing is kept in a cooled place). Before performing the experiment the washing is poured into a syringe of 20-cubic centimeter capacity and carefully filtrated through cotton. The complements for the test are prepared according to the usual method. The hemolytic serum is used in triple titer; the specific influenza antisera are diluted to 75 times less than their titer. Standard influenzal diagnostica A (or A-prime) and B from the allantoic fluid of chick embryos is introduced into the test as a control.

For the purpose of simplifying the technical aspect

of the test 1.2-fold dilutions of complement are prepared directly before the beginning of the experiment (Table 107).

Table 107

Schema of Diluting Complement Using a Dilution Factor of 1.2

No. of test-tube	1	2	3	4	5	6	7	8	9	10
0,85% saline solution	—	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
Complement in 1:5 dilution	12,0	—	—	—	—	—	—	—	—	—
Volume of complement serially transferred from test-tube to test-tube	—	10,0	10,0	10,0	10,0	10,0	10,0	10,0	—	—
Dilutions obtained	1:5	1:6	1:7,2	1:8,6	1:10,3	1:12,4	1:14,8	1:17,8	—	—

These complement dilutions are kept in ice and used on the same day.

Performance of the Preliminary Experiment

Complement Titration

Complement in dilutions of from 1:5 to 1:17.8	0.2 cubic centimeter
0.85 percent saline solution	5.2 " "
Hemolytic system (equal volumes of triple titer hemolytic serum and six-percent erythrocyte suspension combined ex tempore)	0.4 " "
Total volume	5.8 cubic centimeter

The test-tubes are shaken and put into a thermostat at 37°. The results are read after 30 minutes. Complete hemolysis in the test-tube corresponding to the greatest complement dilution indicates the limit of its activity.

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Complement in four ascending doses (1; 1.2; 1.44; 1.72) is poured out in 0.2-cubic centimeter quantities into four series of test-tubes: A serum in a dose of 0.2 cubic centimeter is poured into the first series; B serum in 0.2 cubic centimeter doses is poured into the second; normal serum in a quantity of 0.2 cubic centimeter is put into the third; and 0.85 percent saline solution in 0.2-cubic-centimeter quantities is poured into the fourth. Five cubic centimeters of the 0.85 percent saline solution is added to all the test-tubes.

The test-tubes are shaken up and put into a thermostat for fixing the complement; after an hour, 0.4 cubic centimeter of the hemolytic system is added to them, and they are again put into the thermostat. After 30 minutes, the results are read. Complete hemolysis corresponding to the highest dilution of complement indicates the minimum dose of complement, which is taken as one unit. In the main experiment 1.75 units of complement are used.

The Main Experiment. The test filtered washing is poured into three test-tubes in a quantity of five cubic centimeters in each. To them are added, respectively, 0.2 cubic centimeter of influenza antisera A, B and normal serum. Two-tenths of a cubic centimeter of complement (1.75 units) is added to each test-tube, and they are kept for 18 hours at a temperature of two-four degrees; then, they are kept for 30 minutes at room temperature. The hemolytic system is added to each test-tube in a quantity of 0.4 cubic centimeter in each. It is necessary to have a serum control (serum plus physiological solution) and a control of the specificity of the reaction (immune plus physiological solution) and a control of the specificity of the reaction (immune serum plus influenzal antigen and immune sera plus antigen diluted with washing from the pharynx of a healthy person). After pouring in the hemolytic system the test-tubes are put into the thermostat at 37° for 20-45 minutes, after which the test is read.

According to the data of Yurikas (1952-1953) and Kantorovich (1952-1953), this method is very sensitive and specific, giving about 50 percent correct results in the examination of influenza patients.

Similar results have been obtained by the authors through the use of the test in practice: in comparison with the ordinary schema the use of the new schema made it possible to diagnose influenza 2.2-4.3 times as often and dynamically during the course of the disease.

During the outbreak of influenza in 1952 virus influenza was diagnosed in 21.6-50.7 percent of the patients by this method. In the review works of Smorodintsev and co-authors (1953) data are presented which indicate the high

degree of specificity of the reaction (Fig. 248) and its suitability for diagnosis during an outbreak of influenza A-prime (Fig. 249).

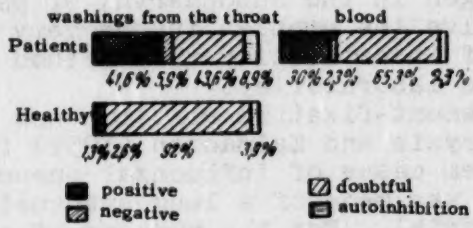

Fig. 248. Data on the Specificity of the Complement Fixation Reaction for the Detection of the Influenzal Antigen (Smorodintsev and others, 1953)

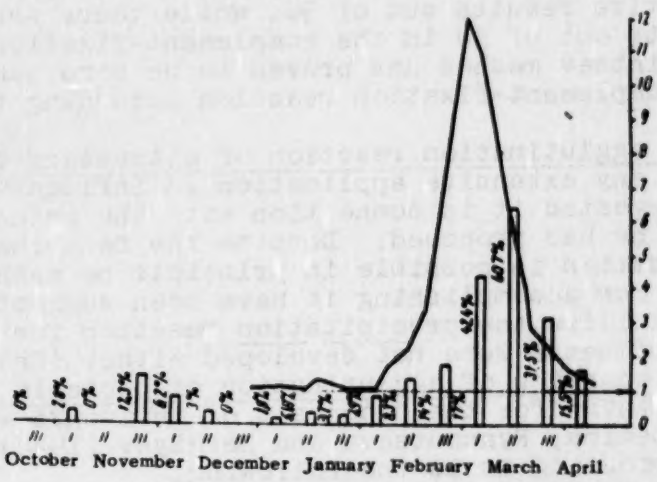

Fig. 249. Influenza Morbidity Rate of the Adult Population (according to five-day periods 1951/1952).

Key: the columns represent the influenzal antigen in the gargling of patients according to the complement-fixation reaction; the curves represent the "zero level" (the average in-

fluenza morbidity rate in the first half of January) (Smorodintsev and others, 1953).

In connection with these data an extensive checking of the diagnosis of influenza is necessary by means of detecting the antigen in the nasopharynx of patients in order to be able to solve the problem in the very near future of the expediency of incorporating this method into the practice of diagnostic laboratories.

The complement-fixation reaction has been successfully used by Dreyzin and Zalmanzon (1954) for the diagnosis of post-mortem cases of influenzal pneumonias. As the test antigen use was made of a lung suspension obtained from autopsy material. For the purpose of eliminating the anticomplementary properties and eliminating the inert substances the authors used freezing and thawing with subsequent centrifugation. The reaction was performed according to the Smorodintsev method. Making use of this method the authors established the influenzal nature of post-mortem pneumonias in a considerable number of cases.

In 1952, in parallel with the complement-fixation reaction the hemagglutination reaction used to be performed with antigens from the lungs; this gave much poorer results (four positive results out of 30, while there were 21 positive results out of 30 in the complement-fixation reaction). The Smorodintsev method has proved to be more sensitive than the complement-fixation reaction according to the usual method.

The agglutination reaction of elementary bodies did not obtain any extensive application in influenza, although Morozov suggested it in connection with the method of viroscopy which he had proposed. Despite the fact that the use of this reaction is possible in principle no methodological approaches for accomplishing it have been suggested. Attempts to utilize the precipitation reaction for the diagnosis of influenza were not developed either (Knight, 1946).

The reaction of agglutination of bacteria loaded with virus (AVB) for the diagnosis of influenza was suggested by Demina, Ryazantseva and Sergiyev (1948). The method of performing it is the following.

Typhoid bacilli (particularly strain No 495 which does not produce spontaneous agglutination) are most suitable as microbe-adsorbents. The microbes are streaked out on slant agar, and after 24 hours a plate subculture is made. The 24-hour culture here is washed with 15 cubic centimeters of physiological solution, and the thick bacterial suspension obtained is heated on the water bath at 58-60° for an hour, diluted 10 times (the undiluted bacterial suspension may be used) with physiological solution or with a

phosphate buffer (pH 6.0-8.0) and mixed with an equal quantity of 10-percent suspension of the lungs of influenza-afflicted mice. The mixture is shaken up for 30 minutes and left for a day in a refrigerator at 4°. The next day, the sediment is washed out three times by means of centrifugation with physiological solution or a phosphate buffer. The washed-out precipitate is diluted to the original volume with physiological solution. A control antigen is prepared in the same way from the lungs of normal mice.

The agglutination reaction is performed according to the usual method, using an ascending dilution of test sera of from 1:10 and higher. Twenty-five hundredths of a cubic centimeter of the appropriate serum dilutions is poured into the test-tubes, and two or three drops of the influenzal antigen is added to each. The serum with the control antigen and the antigen with the physiological solution constitute controls. The test-tubes are shaken up, put into a thermostat for two hours at 37°, and then left at room temperature until the next morning. The intensity of the reaction is designated by the signs ++++, +++, ++, + and -. There should be no agglutination in the control test-tubes.

Checking of the diagnostic value of this reaction was carried out by Kharmats (1949), Vaynberg and Borovkovaya (1953). According to the data of Kharmats this reaction is less sensitive than the hemagglutination reaction. In the hands of Vaynberg and Borovkovaya it produced better results (Fig. 250), which, however, are no better than those of the hemagglutination reaction. It should be kept in mind that in the method used this reaction produces very inconsistent results, in connection with which it can hardly be recommended for extensive practical application.

For the purpose of diagnosing influenza use may be made of the methods of determining the specific toxin in the blood (Zakstel'skaya, 1953). The toxic properties of the blood are determined by the method of tissue constriction after Tarusov, which was mentioned in the second chapter. According to Zakstel'skaya's data, in investigating 81 samples of blood taken during the first two days of the disease 63 tests gave a distinct positive result (77 percent of the clinical diagnoses).

This method is very tedious, however, and can hardly obtain extensive practical application.

Sereda (1952) suggested using changes in the electrokinetic potential of erythrocytes caused by the virus for the diagnosis of influenza. According to this data, the decrease in the erythrocyte electrokinetic potential occurs under the influence of a dose of virus three to four times less than the minimum hemagglutinating dose. On examination the test washing or other material is placed in an electro-

phoresis chamber, and the erythrocyte mobility is determined; it is reduced under the influence of the virus. Neutralization of the virus with specific serum eliminates this effect. The author utilized this method with good results for the diagnosis of influenza in 1952; however, this diagnostic method has not as yet been checked by others.

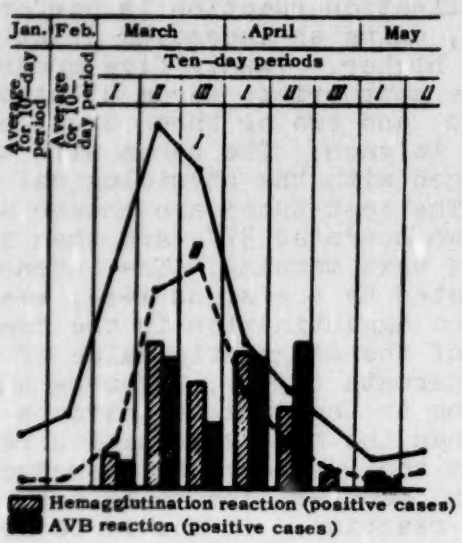

Fig. 250. Morbidity Rate Curves in January-May 1952 For Influenza-Like Forms (I) and Virus Influenza Diagnosed by Clinical Signs (II) in Comparison With Positive Results of Hemagglutination Reaction and AVB reaction.

The scales of the height of the curves and of the columns differ by 150 times (Vaynberg and Borovkova, 1953).

Based on the fact that the influenza virus destroys the nasopharyngeal mucus inhibitors in the acute stage, Fazekas de St. Groth (1952) suggested using the inhibitor titration as a method of diagnosing influenza, expressing it quantitatively in the form of the inhibitor index. According to his data, this index does not depend on the donor,

the quantity of mucus used, or the period of time it is kept, and in normal persons is equal to 33.2 + 7.4 (such accuracy of determination is hardly necessary). Through the addition of negligible quantities of the virus the index is markedly increased. As an example we are presenting the data of titration of the inhibitor index in one patient. During the six days of the disease the index was equal to 100, 193, 71, 28, 25, 35, respectively, and during convalescence, to 34. The virus was isolated from this patient. During the period of convalescence the titer of hemagglutinins and of complement-fixing antibodies increased. It would be premature to evaluate the practical value of this method until it is checked more extensively.

79. Immunologic Methods

Antibody determination in the blood of influenza patients is the main method of immunological diagnosis of influenza.

Beginning with the sixth to seventh day after the onset of the disease influenzal antibodies accumulate in the blood of convalescents; these may arbitrarily be subdivided into neutralysins (antibodies which neutralize the influenza virus in in vitro experiments), antihemagglutinins (antibodies which inhibit hemagglutination) and antibodies which fix complement. This arbitrary division is caused chiefly by the difference in methods of detection. For diagnostic purposes the detection of antibody accumulation dynamically, that is, the determination of the rise in their quantity which is achieved by means of a two-fold examination of the blood, is required.

The first time, blood is taken (with a five-cubic centimeter syringe from the antecubital vein) during the first few days of the disease (no later than the sixth), and the second time, during the recovery period but no earlier than the 14th day and no later than a month after the onset of the disease. For the purpose of taking blood from small children we usually use the following method. We extract 0.1-0.2 cubic centimeter of the blood from the heel and immediately dilute it to 10 times its volume with physiological solution. It is preferable to use physiological solution which has first been poured into conical test tubes, to which the blood is added which had been taken up in a capillary pipette. The mixture is centrifuged; the erythrocytes which fall to the bottom are discarded; the diluted serum is used for the test.

Undiluted serum is heated for 30 minutes at 60° before the experiment. The diluted serum is not heated.

In the practice of laboratory diagnosis of influenza the neutralization reaction in vivo (for detection of neutralysins) is used rarely because of the duration of the experiments and the considerable expenditure of animals. Most frequently the method of inhibition or suppression of the hemagglutination reaction is used, which makes it possible to determine the quantity and the rise in the titer of influenzal antihemagglutinins rapidly and quite accurately.

Sera taken in the acute stages of the disease and after recovery are investigated simultaneously. For the experiment, either diagnostica (influenza virus inactivated with formalin in a dilution of 1:2000) or a live virus suspension of influenza virus of types A, B, C and D is required. For the latter, use is made of allantoic (for the C virus -- amniotic) fluid of infected chick embryos or a lung suspension taken from white mice infected and killed after 48-72 hours. Before the experiment a determination is made of the hemagglutinating unit of the virus suspension, for which it is titrated out with two-percent suspension of washed chick erythrocytes. The highest dilution which produces erythrocyte agglutination is taken as the hemagglutinating unit and is used for the test in a four-fold dose (four hemagglutinating units). Whole serum is diluted 1:10 with physiological solution and is poured out into test-tubes in ascending dilutions, using a two-fold dilution factor. Usually, test-tubes are used which are arranged in four rows, each containing eight tubes (up to a dilution of 1:2560). An equal volume of the A virus (first series of test tubes) B virus (second series) and D (third series) in a dose of four hemagglutinating units is added to 0.25 cubic centimeter of each dilution of the serum taken in the acute period of the disease, after which 0.5 cubic centimeter of a two-percent suspension of washed chick erythrocytes is added to all the test-tubes, into which serum is added taken from the patient after recovery. All the test-tubes are carefully shaken up and left to stand at room temperature. The results of the tests are determined twice, after one and two hours. The method of performing the test has been described above.

A positive reaction and its titer are determined according to the last test-tube, where the final serum dilution still inhibits the erythrocyte agglutination. The difference between the corresponding rows of test-tubes indicates the rise in antibody titer, which is of diagnostic importance.

The complement-fixation reaction is also used for the serum diagnosis of influenza. Serum from the patients is taken at the times mentioned above and is treated by the same

method (heating in a water bath for 30 minutes at 60°). Lung tissue of mice infected with influenza virus and killed after 40 hours is used as the antigen. Finely minced tissue is suspended in 10 volumes of physiological solution and centrifuged at 3,000 revolutions per minute. The antigen obtained may be utilized in the complement-fixation reaction after a simple treatment which consists of freezing once and subsequent centrifugation. Freezing to a considerable degree makes it possible to eliminate the anti-complementary properties which are characteristic of tissue antigens generally. The antigen may be used also without a preliminary freezing under conditions where the original suspension is diluted by no less than 40 times. Lung tissue of healthy mice prepared in a similar way is used as a control.

Extra-embryonic fluid of chick embryos infected with the virus may also be used as the antigen.

Before the experiment titration of complement is accomplished in the presence of antigens. In all subsequent stages of the test the complement is used in no less than two different doses containing 1.5 and 2 minimum hemolytic units. Then, the optimum dose of the antigen is established by means of titration of different dilutions of it with serum known to be anti-influenzal and only after this is the main experiment begun.

The test sera are diluted using two-fold dilutions (from 1:4 to 1:128) and are poured out into test tubes of the same diameter in a volume of 0.2 cubic centimeter. Two-tenths of a cubic centimeter of antigen and 0.2 cubic centimeter. Two-tenths of a cubic centimeter of antigen and 0.2 cubic centimeter of complement are added to the serum. Two test tubes with the test serum in dilutions of 1:4 and 1:8 (for the detection of autoinhibition) and two test-tubes with the same dilutions containing antigen from normal tissue serve as controls. The complement-fixation reaction is continued for one hour at a temperature of 37° for 18 hours at a temperature of 4-5°. Then, 0.4 cubic centimeter of the hemolytic system is added, consisting of three-percent suspension of sheep erythrocytes sensitized by the hemolytic serum. Reading of the results of the experiment is carried out after 30-40 minutes (depending on the procedure used for the controls) of keeping the test-tubes at 37°. The complement-fixing antibodies are determined with the corresponding influenza A and B viruses. A positive serological diagnosis is established by the difference in titers between the first (early) and second (late) serum taken from each patient. An increase in the antibody titer of four or more times is of reliable diagnostic significance.

The methods listed for retrospective laboratory diagnosis of influenza are of definite importance for checking the correctness of early diagnosis. The detection of antihemagglutinins in the blood of patients is simpler to accomplish than the other tests, is sufficiently exact and is entirely suitable for practice with respect to solitary and massive investigations. In the case of influenza in children their low capacity of forming antibodies under the age of one month should be kept in mind. As the child grows older this capacity gradually increases. Thus, for example, according to material available to us, of the group of 336 children from six months to two years of age who had had influenza A and B a rise in antibody titer (antihemagglutinins) of four or more times was noted in 94 percent (Ritova, 1951).

While the complement-fixation reaction has a certain complexity in its application it has the advantage that it does not possess strain-specificity in differentiating influenzal A and B antibodies. This means that its range of sensitivity is greater, and it includes all the strains of one type of virus. Experience in serological diagnosis shows that while it is sufficient to use a single virus strain for the complement-fixation reaction which will detect all the antibodies of the same type, the detection of antihemagglutinins not uncommonly depends on the correlation of strain used in the test and the antibodies of the given blood serum. It should also be taken in consideration that the complement-fixing antibodies in influenza appear earlier in the blood than the neutralysins and antihemagglutinins.

We have presented a description of the most generally used methods of performing the hemagglutination-inhibition reaction and the complement-fixation reaction. Without giving a detailed description of the results of application of these tests for the diagnosis of influenza we should like to present only certain results of investigations which have been carried out chiefly in recent years.

The reliability of the hemagglutination-inhibition reaction as a method of serological diagnosis of influenza was checked in the Soviet Union during an epidemic of influenza A in 1943/44, at which time a correlation of the results obtained through the use of hemagglutination-inhibition reaction and the complement-fixation reaction was obtained (Nartsissov and Parnes, 1948). In Germany, the etiologic role of the A virus was established by the same method during an outbreak of influenza in the winter of 1948/49 (Hausmann and others, 1950). At the same time, an outbreak of influenza A in the USSR was studied in Moscow by Solov'yev (1950), and in Khar'kov, by Lushkina (1950). The

latter showed that the serological changes in the blood of the population (during large-scale investigations of donor blood) closely follow the movement of the influenza morbidity rate and are absent in the seasonal rises in the incidence of acute catarrhs (Fig. 251). During the three years of the observations there were two outbreaks of influenza A-A-prime in the city -- in February 1947 and in April 1949 -- and both times a definite increase in the antibody titer against influenza A occurred in the population. During this time interval two seasonal rises in morbidity rate with acute catarrhs occurred: from October 1947 through March 1948 and from October 1948 through March 1949, which were not accompanied by any pronounced increase in the antibody level against influenza in the population. There were no outbreaks of influenza B during this period. A more detailed analysis made it possible to detect moderate seasonal rises in the morbidity rate for influenza A and B (November-February) and an intense outbreak of influenza A (March-April) from September 1948 through June 1949; this latter was afterwards diagnosed as an outbreak of influenza A-prime (Fig. 252). Similar rules and regulations were established with respect to influenza B in the winter of 1949-50 by Solov'yev and Zakstel'skaya (1951).

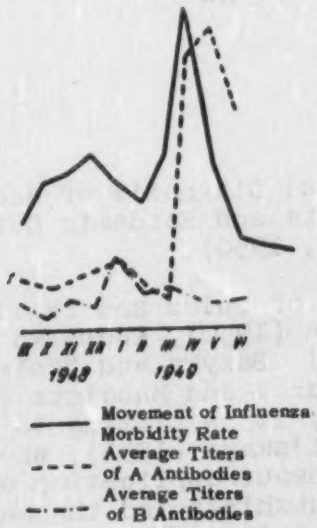

Fig. 251. Influenza and Catarrh Morbidity Rates and Serological Changes in the Blood of the Population (Lukshina, 1950).

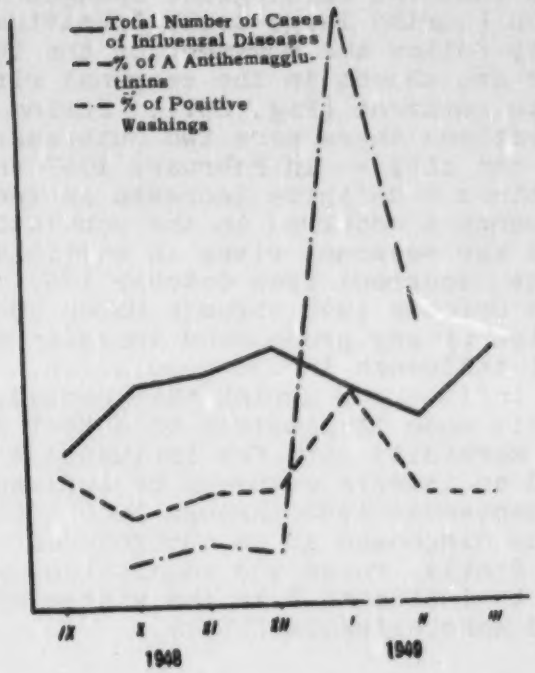

Fig. 252. Serological Diagnosis of Seasonal Rise in Morbidity Rate and Epidemic Outbreak of Influenza (Lukshina, 1950).

The same kind of rules and regulations were noted in the works of Peterson (1950), Khomenko and co-authors (1952, 1953), Shikina (1952), Bazyka and Ovsiyevskaya (1952), Rappoport (1953), Shindarov and Manolova (1954).

In conclusion, it is fitting to present the data of Solov'yev and Zakstel'skaya (1954), who showed the expediency of the simultaneous application of the hemagglutination reaction with washings and the hemagglutination-inhibition reaction with paired sera. As seen from the graph presented (Fig. 253), the proper utilization of this method makes it possible to obtain quite an accurate idea of the nature of influenzal diseases. By making use of this method of analysis, Zhdanov and Lukshina (1950) were able to extrapolate the results of the investigations and compute the city-wide morbidity rates for influenza and acute catarrhs

(Fig. 254). Despite a certain schematism of the data obtained, they give us an idea of the quantitative aspect of the epidemic process in influenza.

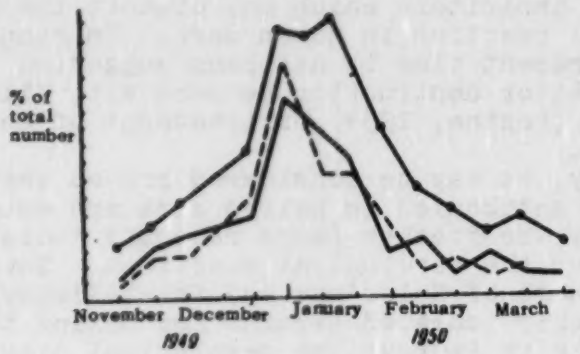

Fig. 253. Distribution (By 10-Day Periods) of Positive Reactions for Influenza in the Blood Sera and in the Washings.

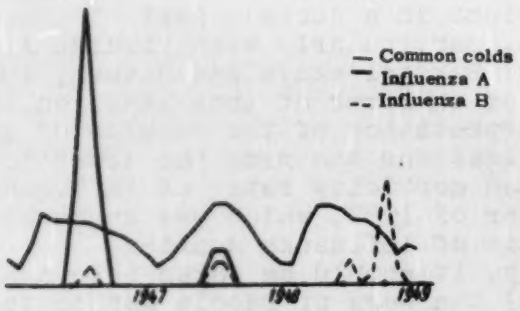

Fig. 254. Movement of Influenza A and B and Common Cold Morbidity Rates (Zhdanov and Lukshina, 1950).

Here, it remains only for us to bring to mind certain characteristics of the serological reactions in the use of the given method, which were analyzed in detail in preceeding chapters.

First, it is essential to keep in mind the presence of nonspecific inhibitors which may distort the nature of the serological reaction in human sera. In connection with this, at the present time it has been suggested that the method of inhibitor destruction be used with filtrates of cholera vibrio (Lepine, 1954) or treatment of the sera with carbon dioxide.

Secondly, it may be considered proved that the changes in the antibodies in paired sera are established more distinctly the fresher (more recently isolated) the strains used for the serological reactions. Thus, for example, in the work of Solov'yev and Zakstel'skaya (1954) the use of freshly isolated strains for making the diagnosis made it possible to improve the serological diagnosis of the disease markedly. Ritova (1954) came across a similar phenomenon recently; during a typical outbreak of influenza in a children's group she was not able to find any rise in antibody titer in paired sera when carrying out the test with standard strains of A, A-prime and B viruses. At the same time, when she performed the reaction with the virus of influenza A-prime isolated from the patients during the outbreak a distinct rise in the antibody titer was determined in the paired sera.

Thirdly, the existence should be kept in mind of inadequate reactions in a certain part of the patients who have influenza, particularly with viruses A and A-prime (Zhdavov, 1954; Sakstel'skaya and Ritova, 1954). Dobrova (1954) committed an error of this kind; on the basis of an incorrect interpretation of the results of large-scale serological investigations she made the assertion that an outbreak (increased morbidity rate) of influenza A had developed in the winter of 1952, which was supposed to have preceded the epidemic of influenza A-prime.

Fourthly, it should be taken into consideration that (in rare cases) the sera of people may contain substances which cause erythrocyte agglutination. Skurskaya (1951), directing attention to this phenomenon, points out that in these sera hemagglutinins against chick erythrocytes are contained but not against other birds.

The complement-fixation reaction for the determination of the rise in antibody titer was studied by Nartsissov (1948) and recently by Kantorovich (1951), Kaganskaya (1952) and others according to the method of Smorodintsev (1952). According to the data of these authors, this reaction was more sensitive than the hemagglutination-inhibi-

bition reaction (by approximately 1 1/2 times). In general, the application of this reaction gives results which are similar to those of the hemagglutination inhibition reaction. In Fig. 255, taken from the work of Smorodintsev and co-authors (1953), data are presented concerning the efficacy of application of this reaction for the diagnosis of an outbreak of influenza A-prime at the beginning of 1952. As seen from the Figure the frequency of positive reactions well reflects the course of the epidemic wave.

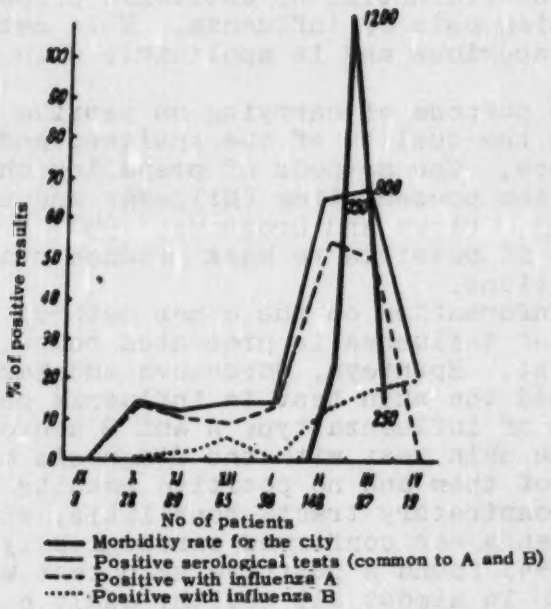

Fig. 255. Influenza Morbidity Rate and Serological Reactions in the Population (Smorodintsev and others, 1953).

The comments which have been made above with respect to interpreting the hemagglutination reactions are to be a considerable degree applicable to the complement-fixation reaction. Apart from the means of improving the method of

this reaction indicated above, it has been usggested that a soluble influenza virus antigen be used as the antigen (Siegert and others, 1954). It is still not clear, however, how much this suggestion improves the methods of performing the reaction.

Of the other serological reactions (the neutralization reaction in laboratory animals has been described above) the conglutinating complement absorption test (Stoker and Marmion, 1950) and the agglutination reaction of collodium particles loaded with influenza virus (Boand and Kempe, 1951) have been suggested; however, they have no advantages over the tests described above. Zakstel'skaya (1953) used a determination of antitoxic properties of the sera for the diagnosis of influenza. This method is complicated and laborious and is applicable only to special investigations.

For the purpose of carrying on massive serological investigations the quality of the antigens and of the sera is of importance. The methods of preparing them have been worked out at the present time (Hilleman and co-authors, 1951; Yeremeyev, 1953, Orlova and Drozdova, 1954; Brand and Müller, 1954) and make it possible to mass produce standard diagnostic preparations.

Brief information on the other methods of immunological diagnosis of influenza is presented below:

Skin Test. Epshteyn, Gorbunova and Karakuyumchan (1954) performed the skin test in influenza patients using virus antigens of influenza type A and B and obtained a correlation of the skin test with the diagnosis of influenza in 60 percent of them and no positive results in acute catarrhs of the respiratory tract, tonsilitis, etc. The diagnosis of influenza was confirmed serologically. Beveridge and Burnet (1944) found a positive skin test with the influenza antigen in almost all healthy adult persons and they believe it is evidence of influenza suffered in the past. In contrast to these data, Epshteyn and co-authors were unable to obtain any positive skin test in a number of persons who had had influenza, in whose blood there were antibodies in various titers at the time of performing the test. Because of the lack of correlation between the data of Soviet and Australian authors the problem of the mechanism of the skin test still remains unsolved.

Gudkova (1952, 1953) studied the phagocytic reaction in impressions of tonsils and in the peripheral blood of influenza patients and patients with acute catarrhs of the respiratory tract. She notes that the phagocytic reaction in influenza patients is markedly reduced during the first few days of the disease and may be completely absent. With the improvement in the course the phagocytic reaction in-

creases in activity. In acute catarrhs of the respiratory tract there is no reduction in the phagocytic reaction.

During an outbreak of influenza A-prime in 1952 Zakstel'skaya (1954) investigated the phagocytic reactivity of the blood in 25 patients.

For the reaction she made use of a fresh 24-hour agar culture of staphylococcus diluted by means of a standard 2,000,000,000 per cubic centimer. Before the experiment the staphylococcal culture was kept for 30 minutes in a thermostat at 37°; 0.2 cubic centimeter of blood taken from the finger was mixed with 0.1 cubic centimeters of a two-percent sodium citrate solution which had been heated to 37°, and 0.1 cubic centimeter of the staphylococcal suspension was added to it. The mixture was shaken up actively and put into a thermostat for 30 minutes. Smears were made from the blood according to the usual method, fixed with methyl alcohol and stained with 0.5-percent alkaline methylene blue or according to the Romanowsky-Giemsa method. Then, a count of 100 neutrophils was made in the smear and the percentage of those phagocytizing the staphylocci was computed. In addition, the intensity of the phagocytosis was taken into consideration, for which the absolute number of phagocytized microbes was divided by 100 (opsono-phagocytic index).

The diagnosis of influenza was confirmed serologically in 16 patients; in 14 it was A-prime; in two, B. As a control 16 healthy persons were examined. Zakstel'skaya notes that both the percent of phagocytizing leucocytes and the value of the phagocytic index were much lower in influenza patients than in healthy persons. Through the dynamic examinations of sever l patients it was shown that the phagocytic activity returned to normal in them six to ten days after the beginning of the disease, and in one patient with antral sinusitis it did not return to normal even after 15 days. Therefore, it may be supposed that activation of conditionally pathogenic microflora in influenza, which produces secondary complications, is the result of a deterioration in the immunobiological condition of the body and a suppression of its defense reactions, if we judge by the depression of the phagocytic activity in influenzal complications.

According to the observations of Leshchinskaya (1955) (69 influenza patients, 26 patients with acute catarrhs of the respiratory tract and 25 control, healthy persons), a considerable depression of phagocytic activity is observed in influenza but not always from the beginning of the disease. Sometimes it occurs only at the time of convalescence. The recovery of the normal phagocytic reaction occurs slowly; sometimes it does not occur for two weeks. As far as acute catarrhs of the respiratory tract are concerned, in cases

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that are uncomplicated and which do not have any associated chronic diseases the phagocytic reaction is not inhibited. Not uncommonly, the depression of the phagocytic reaction was noted in persons suffering from associated chronic diseases of the ENT organs.

Therefore, this method requires further study, needs to be made more exact, and dynamic observations and confirmations on a larger collection of material are necessary before this test can be included in general practice for the differential diagnosis of influenza and acute catarrhs of the respiratory tract.

In evaluating the methods of laboratory diagnosis of influenza described it should be recognized that they are not all equivalent (Smorodintsev, 1948, 1949; Smorodintsev and others, 1953; Lepine, 1954).

With respect to the reliability of the results obtained serological methods of investigation come first (determination of the antibody dynamics in the hemagglutination-inhibition and complement-fixation reactions). At the same time, in practice the most valuable methods are those methods of early diagnosis which are not so reliable -- determination of the antigen in the nasopharynx (in the hemagglutination and complement-fixation reaction) and clinical-laboratory methods -- cytological investigations of impressions taken from the nasal mucosa and white blood examination.

80. Differential Diagnosis of Influenza and Other Acute Febrile Diseases

The diagnosis of influenza is very difficult. Many physicians erroneously take almost any febrile disease for influenza during the first few days of the disease, particularly if it is associated with the toxic state and catarrhal signs.

Hence, the need arises for making a differential diagnosis between influenza and many infectious diseases. The differential diagnosis of influenza from acute catarrhs of the respiratory tract is most difficult (see Smorodintsev, Kofman and others, 1953). On the basis of the works of Smorodintsev, Kolyaditskaya, Korovin, Nechayev, Rossiyskiy, Levinson and Epshteyn the Commission on Influenza Control of the Scientific Medical Council of the Ministry of Health RSFSR constructed a Table for the differential diagnosis between influenza and common cold-type catarrhs. However, we cannot agree completely with certain differential diagnostic features shown in this Table (Zhurn. Mikrobiol., Epidemiol. i Immunobiol., 1943, Nos 10-11). For example, herpetic eruptions in influenza are not necessarily less common than in

acute catarrhs, and the duration of the acute signs in the latter was no greater than in influenza. True, in acute catarrhs we observed chronic pathology in the upper respiratory tract much more often than in influenza, as well as in the throat, nasal sinuses, and ears.

Therefore, the disease cannot always be considered terminated with the elimination of the acute signs, although it is known that many patients during this period return to work. Chronic pathology which remains in influenza and in acute catarrhs of the respiratory tract require the application of special measures. We are presenting a Table of characteristics which we recommend in diagnosing influenza and acute catarrhs of the respiratory tract; it was made with consideration of the main principles of etiology, epidemiology, clinical aspects and laboratory examinations (Table 108).

Table 108

Differential Diagnosis of Influenza and Acute Catarrhs of the Respiratory Tract

No of feature	Features	Influenza	Acute Catarrhs
	Etiology		
1	Pathogen	Specific Influenza Virus type A, A-prime, B, C and D	Not clarified yet; frequently the microbial flora is significant. Certain forms are caused by viruses
2	Chilling factor	Not of decisive importance	Is fundamental in the majority of cases
3	Immunity	Formed	Not formed
	Epidemiology		
1	Spread	Rapid including large groups, sometimes a number of cities and countries	Depending on weather conditions, sometimes in outbreaks

Table 108 [continued]

No of feature	Features	Influenza	Acute Catarrhs
2	Duration of outbreak	Brief with rapid rise and abrupt fall in morbidity rate	Regular seasonal fluctuations depending on the weather
3	Climatic characteristics	Do not solve the problem	Of great importance
4	Contagiousness	To a high degree	None in adults with cold-catarrhs. In children there is a contact danger. The acute catarrh viruses are contagious
4	Focalization	Frequent	Rarely observed
	Clinical Aspects		
1	Onset	Sudden with chill, usually without prodromal signs	Gradual
2	External appearance of the patient	Puffiness of the face, hyperemia of the face and conjunctivae	Ordinary
3	Main symptoms	General intoxication	Signs of catarrh of the respiratory tract
4	Toxic State	Pronounced	Absent or slight
5	Catarrhal signs	May not be observed	Main symptoms
6	Headache, depression, adynamia	Marked	Moderate or absent
7	Fever	Usually high	Usually low-grade
8	Pains in eyes, conjunctivitis	Usually present	Absent or rare
9	Hypotension	Present	Not necessary

Table 108 [continued]

No of feature	Features	Influenza	Acute Catarrhs
10	Tendency toward perspiration	Frequent from beginning of disease	Less often observed
11	Coryza	Nose blocked. Coryza not uncommonly appears on the second to third day of the disease	Intense from very beginning of disease
12	Cough	Inconstant symptom. Dry, hacking.	Cough intense, often productive
13	Complications	Frequent, chiefly in the respiratory tract	Rare
14	Recurrence of disease	Infrequent	May be frequent
	Laboratory Data		
1	Blood	Leukopenia beginning with the second or third day, frequently with the first day of the disease. Absence or reduction in eosinophil count, neutropenia, relative lymphocytosis, monocytosis, toxic granulation of neutrophils, thrombocytopenia. The sedimentation rate is usually not accelerated.	Usually, normal leukocyte count or slight leukocytosis. The sedimentation rate is often accelerated. No thrombocytopenia is observed.
2	Hemagglutination reaction with throat washing	Often positive	Negative
3	Rhinocytoscopy	Presence of columnar epithelial cells during the first few days of	Solitary columnar epithelial cells may be observed. In chronic nasal

Table 108 [continued]

No of feature	Features	Influenza	Acute Catarrhs
		the disease in impressions taken from the inferior nasal concha, sometimes inclusion bodies	disease they are noted in approximately 40 percent of the cases.
4	Rise in antibody titer against influenza virus	Observed in the great majority of cases	Not seen

As seen from the material presented, we are not helpless in the differential diagnosis of influenza and acute catarrhs. Stuart-Harris (1941) points out correctly also that the retrospective diagnosis as well as the establishment of the diagnosis of the disease in a whole group of persons is much easier than the diagnosis of the disease in each individual case. In addition to the characteristics of the course and the symptomatology, the epidemiological history and methods of laboratory diagnosis are of great help in the diagnosis of influenza.

Typhus. Serious difficulties arise in the differential diagnosis of influenza from typhus at the beginning of the disease. On the fifth-sixth day it is easier to distinguish influenza from typhus: in typhus there is usually a definite rash, while in influenza the majority of patients have returned to work by this time.

The appearance of the patients and the reactions of the vascular and nervous systems in these diseases are very similar.

On the basis of personal experience we have become convinced of the value of certain statements made by Braude and Linetskaya (1946) with respect to the differential diagnosis of influenza and typhus. In influenza the parasympathetic functions of the vegetative system predominate over the sympathetic functions. This is expressed, for example, in the condition of the skin (appearance of red dermographis, increase in perspiration), of the pulse (not uncommonly there is bradycardia), blood (leukopenia), etc. In typhus, on the other hand, the sympathetic functions predominate over the parasympathetic (dryness of the skin, white dermographia, tachycardia, leukocytosis). This should be kept in mind in

evaluating the symptoms which are observed in these diseases.

In a number of cases a carefully taken history, particularly epidemiological history, aids in clarifying the diagnosis.

Even greater difficulties arise in the differential diagnosis of influenza and recurrent typhus. The latter, like influenza, may be associated with catarrhal signs, tendency toward perspiration, bradycardia, absence of an enlarged spleen leukopenia. The diagnosis of the disease is still further complicated when a negative Weil-Felix test and a negative test with the Rickettsia prowazeki are obtained in the absence of a suggestive epidemiological history and in the presence of the indistinct symptomatology. In such cases the appearance of the characteristic rash and the negative virological and rhinocytoscopic examinations come to the rescue, although after some delay, and make it possible to rule out influenza and make the diagnosis of re current typhus.

In Table 109 the differential-diagnostic features between influenza and typhus are given.

Table 109

Differential Diagnosis of Influenza and Typhus

No of feature	Feature	Influenza	Typhus
1	Onset	Usually sudden, without prodromal signs	Usually there are prodromal signs
2	Temperature rise	Rapid, frequent with maximum temperature on the first day of the disease	Usually not so rapid with temperature increasing somewhat up to the second or third day
3	Temperature	Usually remittent	Usually constant
4	General condition	Usually sluggish, apathetic	Usually restless, excited
5	Headache	Front part of the head	Whole head
6	Skin	Tendency toward perspiration	Dry

Table 109 [continued]

No of feature	Feature	Influenza	Typhus
7	Herpes	Not uncommonly	As a rare exception
8	Dermographia	Usually red, rarely white	Usually white
9	Catarrhs of the upper respiratory tract	Usually rhinopharyngitis, often laryngotracheitis and bronchitis	Catarrhal signs of the upper respiratory tract are less frequent
10	Throat	Usually hyperemic and edema of the soft palate and uvula	Enanthem, hyperemia of the soft and hard palates
11	Tongue	Frequently unchanged	Dry, wide, passively dropping margin; frequently positive Godelier sign
12	Spleen	Usually not enlarged	Enlarged
13	Eyes	Usually conjunctivitis	Conjunctivitis and scleritis
14	Pulse	Not uncommonly, relative bradycardia	Tachycardia
15	Tremor	Usually absent	Frequently tremor of the hands and tongue
16	Tourniquet sign	Negative	Positive
17	Rhinocytoscopy	Columnar epithelium, sometimes in layers	No columnar epithelium or rare solitary cells noted
18	Blood	Usually, leukopenia, lymphocytosis, monocytosis, slight shift to the left, Türk cells are rare or absent, normal sedimentation rate, moder-	Less often, leukopenia, usually neutrophilia, lympho penia, considerable shift to the left, Türk cells, accelerated sedimentation rate,

Table 109 [continued]

No of feature	Feature	Influenza	Typhus
		ate decrease in thromboycte count	marked thrombocytopenia
19	Hemagglutination reaction	May be positive	Negative

Primary Atypical Pneumonia. It is usually impossible to diagnose this pneumonia clinically; it is found only by X-ray.

In primary atypical pneumonia of the interstitial type it is clinically impossible to find any changes in the lungs in the beginning of the disease. Moist rales are detected only after several days or at the end of the disease.

Primary atypical pneumonias are also distinguished by the fact that they are resistant to penicillin and to sulfanilamide.

For the purpose of diagnosing primary atypical pneumonia use is made of the "cold agglutination" serological reaction, which consists of the mixing of a two-percent suspension of human erythrocytes, group I, with the patient's serum. The mixture of serum and erythrocytes is allowed to stand for 12-24 hours at a temperature of 2-10°. Streptococcus MG agglutination is also used.

In the great majority of cases (62 percent), primary atypical pneumonias progress, according to the data of Dingle and co-authors (1945), with a leukocyte count of from 6,000-10,000. In 27 percent of the patients the leukocyte count is even higher, and in the other cases a leukopenia is noted. The number of neutrophils reaches 70 percent. The sedimentation rate, according to the data of Kneeland and Smetana (1940), is accelerated. According to Longcope (1940), a slight increase in the eosinophil count (from one to seven percent) and a shift to the left down to the myelocyte level is noted in 18 percent of the patients. The blood picture, the data of the X-ray examination as well as the cold agglutination reaction distinguish this disease from influenza.

Croupous Pneumonia. Patients with croupous pneumonia, particularly central, frequently are taken for influenza patients. This is explained not only by the similarity of the external appearance of these patients (hyperemia of the face and eyes) but also by the possible absence of any severe

dyspnea as well as of percussion or ausculatory changes in the lungs during the first few days of formation of this pneumonia. Repeated X-ray examination, which confirms the existence of a lung infiltrate, and the examination of the white blood with the detection of leukocytosis from the very beginning of the disease assist in clarifying the diagnosis.

Croupous pneumonias with a normal or even a reduced leukocyte count are very rarely encountered. These pneumonias, which are accompanied by leukopenia, usually occur in old people. The severe cause of the disease is usually associated with an areactivity of the bodies of such patients. The prognosis of the disease is poor.

Psittacosis and Ornithosis. Since it begins acutely -- with a high fever and chill, headaches and muscular aches, depression, rheumatic-type aches in the lumbar area, adynamia, conjunctivitis, and vomiting -- psittacosis is sometimes erroneously taken for influenza. The following features of the disease which are not characteristic of influenza throw doubt on the correctness of this diagnosis: irritability, excitation, insomnia, enlargement of the liver and spleen, normal or increased blood pressure, accelerated sedimentation rate, sometimes vomiting, diarrhea in the presence of lung changes (pneumonia), at times very severe.

The intracutaneous test worked out by I. I. Terskikh (1954, 1955), which gives a positive result with the psittacosis (or ornithosis) antigen as early as beginning with the third or fourth day of the disease, in addition to the negative date with respect to influenza, solves the problem on behalf of a diagnosis of psittacosis (or ornithosis). In retrospect, the diagnosis may be confirmed by the demonstration of the rise in antibody titer through the method of complement-fixation performed with paired blood sera of the patient taken at the beginning of the disease and during recovery. This method, like the isolation of psittacosis (or ornithosis) virus strains from the sputum and blood of the patient, is very tedious, laborious and a somewhat delayed examination of specific nature.

"Q" Fever. The differential diagnosis between influenza and "Q" fever is particularly complicated when the latter occurs in an abortive form. Marked toxic and other signs in "Q" fever -- chill, headache, increased temperature, depression, rheumatic pains, muscular aches, pains in the eyeballs, hyperemia of the face and conjunctivae, brachycardia, tendency toward perspiration, catarrhal signs and leukopenia -- very much resemble thepicture of influenza.

Repeated chills accompanied by profuse perspiration, enlargement of the spleen and liver or, according to the data of Leshchinskaya (1953), pains and tenderness on palpa-

tion in the epigastrium and in both subcostal areas, as well as a normal blood pressure speak against the diagnosis of influenza. The final diagnosis is made on the basis of an antibody rise against the Rickettsia burneti and the negative virological and serological investigations for influenza. At the present time, a positive allergic skin test with the Rickettsia burneti antigen is being studied.

Diseases of the Typhoid-Paratyphoid Group. Patients with diseases of the typhoid-paratyphoid group are distinguished from influenza patients by their external appearance. They have no puffiness or hyperemia of the face, eyes or other ocular symptoms as in influenza. The temperature increases gradually. The tongue is markedly coated, the intestine is distended, there are borborygmus and sometimes pains in the right iliac area and enlargement of the spleen. A "typhoidal" state is characteristic of them: insomnia with pains in the entire head, delirium, nightmares, sometimes from the very beginning of the disease. Blood culture in bile produces the growth of typhoid-paratyphoid bacilli. Rhinocytoscopy, hemagglutination and other laboratory tests typical of influenza are negative. By the time of appearance of roseola in the patient with a typhoid-paratyphoid disease, or by the time the Widal reaction becomes positive, attesting to the climax of the disease, the patient with uncomplicated influenza usually has recovered.

Toxoinfections. A toxic state and catarrhal signs of the upper respiratory tract give us the grounds for confusing toxoinfection (salmonellosis) with influenza. The epidemiological data (simultaneous occurrence in several persons eating in the same dining room, chiefly in the summer) as well as the occurrence of gastrointestinal disorders in the patients (vomiting, diarrhea, abdominal pains), sometimes accompanied by a rash, jaundice, enlargement of the liver and spleen, neutrophilia and acceleration of the sedimentation rate (Tsygankov, 1950) in the absence of laboratory data speaking for influenza solve the problem on behalf of the diagnosis of toxoinfection.

Dysentery. Patients with dysentery are sometimes taken for influenza patients before the occurrence of the typical dysenteric stool on account of the signs of the toxic state during the first few days.

Coprological and microbiological examination as well as the data of rectosigmoidoscopy confirm the diagnosis of dysentery.

Tuberculosis. Sometimes influenza is confused with pulmonary and non-pulmonary tuberculosis, frequently with tuberculosis of the lymphatic nodes assuming the mask of influenza (Levenshteyn, 1938; Magazinik, 1932). Tuberculosis should be kept in mind particularly in cases of so-called

"frequently relapsing influenza".

Outbreaks of tuberculous infiltrative pulmonary processes or of bronchoadenitides, accompanied by general toxic (fever, headache, depression, muscle and nerve pains, general weakness, sluggishness, loss of the ability to work, tendency towards perspiration) and sometimes catarrhal signs may imitate influenza.

In tuberculosis the temperature usually rises gradually or is at a low-grade fever level for a long time. The toxic state is not so marked. The patient is euphoric and frequently able to work. The sedimentation rate is accelerated, and there is a moderate leukocytosis in the blood. In tuberculosis the catarrhal signs are less pronounced than in influenza. The data of the history, which indicates contact with a tuberculous patient, the changes in the lungs, the laboratory (sputum analysis by all the latest methods) and X-ray investigations aid in establishing the diagnosis.

With respect to the allergic skin test it should be kept in mind that an influenzal infection leads to anergization of the body, which may be accompanied by the disappearance of the Pirquet reaction, etc.

In miliary tuberculosis there is usually dyspnea, cyanosis, tachycardia, enlarged spleen and liver, which are rarely observed in influenza. Investigation of the optic fundus with a search for tubercles in the retina, a positive diazoreaction in the urine, and X-ray of the lungs contribute to clarifying the diagnosis.

Patients with meningitis (cerebrospinal, tuberculous) measles and a number of other infectious diseases have been sent to the hospital with the diagnosis of toxic influenza. We should like to present brief differential -- diagnostic signs of these diseases.

Epidemic Cerebrospinal Meningitis. The sudden onset of disease, a toxic state and catarrhal signs in the nasopharynx and pharynx give us the grounds for associating the disease with an influenzal infection, particularly since vomiting, meningeal signs, tachycardia and other symptoms characteristic of meningitis may also be observed in influenza. However, some of them should give the physician the idea of the existence of the latter disease. These symptoms are marked headache, excitation, hyperesthesia of the skin, repeated vomiting, convulsions, rigidity of the occipital muscles, retraction of the head, clouded consciousness, detection of pathology in the cranial nerves, Babinski, Kernig, Brudzinski and other signs, the inequality of the tendon or skin reflexes, monoparesis and hemiparesis, white dermographia, pyramidal signs, rash and leukocytosis. The diagnosis is confirmed by examination of the spinal fluid (turbidity, presence of pus cells, chiefly neutrophils).

Pneumococcal Meningitis. Pneumococcal meningitis has the same course as meningococcal meningitis. The diagnosis is clarified only through a bacteriological examination of the spinal fluid.

Tuberculous Meningitis is distinguished by a slower, that is, less stormy course. Usually, it begins with an involvement of the oculomotor nerves. The subjective and objective symptoms may not be so pronounced; however, once they appear they gradually increase. The investigation of the optic fundus sometimes assists in clarifying the diagnosis. On lumbar puncture, the spinal fluid is transparent or slightly opalescent, as a rule, with a predominance of lymphocytes, reduction in the sugar concentration and a high protein concentration. On standing it forms a delicate web-like net -- the fibrin pellicle. Tubercle bacilli may be found in the fluid. This is the symptomatology which distinguishes meningitis from influenza.

Epidemic Seasonal Encephalitis. During the first few days of the disease it may be confused with influenza.

In diagnosing encephalitis it is important to take into consideration the epidemiological history (persons are afflicted who work in the taiga, chiefly from May through August -- the latter statement refers to seasonal encephalitis) as well as the localization and form of the process, on which the principal symptomatology depends.

Along with the general signs (headache, vomiting, sleep disturbance, dizziness, temperature rise, etc.) general cerebral symptoms, meningeal signs, spastic paralysis, oculomotor disorders, diplopia, ptosis of the lids, hyperkinesias, nystagmus, and increase in the tendon reflexes predominate. Sometimes, increased salivation, rash (rarely herpes) are noted with disorders of consciousness and of the psyche. The blood shows a leukocytosis, neutrophilia, lymphopenia with a shift to the left, irritation cells, and an accelerated sedimentation rate. The conglomeration of these signs speaks against influenza, which usually lasts a total of several days.

Poliomyelitis. Fever, headache, catarrhal signs of the upper respiratory tract, rigidity of the occipital muscles and hyperesthesia frequently precede the occurrence of paralysis. At this stage of the disease poliomyelitis is confused with influenza. The occurrence of gastrointestinal disorders (diarrhea or constipation) and of various symptoms of the nervous system (hyperesthesia of the skin and muscles on palpation, twitching of the muscles, weakness in the extremities, pains along the spine, reduction in the skin and tendon reflexes, meningeal signs, sometimes of an erythematous-exudative nature) speak against influenza. With the onset of paralysis of various muscle groups it is hardly pos-

sible to think of influenza. The epidemics are of a seasonal nature (summer-autumn). Usually, children under five years of age become sick with it.

Botkin's Disease [Infectious Hepatitis]. During the prodromal period of epidemic hepatitis fever, chilly feelings, general weakness, sometimes pains in the extremities and joints, catarrhs of the upper respiratory tract may occur which give the impression of being influenza. An increase in the general weakness, occurrence of dyspeptic disorders, poor appetite, nausea, vomiting, distention of the abdomen, sensitivity or pain in the right subcostal area or in the epigastric region, enlargement and tenderness of the liver, and sometimes of the spleen, as well as an increase in the concentration of blood bilirubin and of aldolase, the activity of which is increased in Botkin's disease, speak against influenza. With the occurrence of icterus of the sclera, and skin, dark urine, and decolorized stool nobody would be able to consider influenza.

Measles. The acute onset and catarrhal signs give us the grounds for confusing cases of measles with influenza at the beginning of the disease. However, in measles the catarrhal signs are marked, there is a conjunctivitis, edema of the lids, photophobia and lacrimation. The temperature rises at the time the rash appears. The diazo-reaction of the urine may be positive. The finding of Filatov-Koplik spots on careful examination of the oral mucosa indicates measles. If the diagnosis is not made in the first few days of the disease, the appearance of the rash on the fourth day of the disease solves the problem. However, such a diagnosis is delayed. In the epidemiological respect it is important to make clear whether there have been any cases of measles in the vicinity of the patient.

Scarlet Fever. The sudden onset, rapid rise in temperature, facial hyperemia, catarrhal lesions in the pharynx, and vomiting constitute the basis for mistaking scarlet fever for influenza at the beginning of the disease, particularly in the mild and abortive forms of the disease. The appearance of the patient is characteristic of scarlet fever: the nasolabial triangle is prominent (pallor of the skin of the nose, chin, around the mouth, against the background of the hyperemic cheeks and the bright red lips). The flaming red throat of the patient and the enlarged submaxillary lymph nodes, the positive tourniquet sign and pinch sign and the occurrence of a finely punctate rash attract attention. The tongue which is at first coated becomes raspberry-colored beginning with the third to fourth day and has hypertrophied papilae. The blood shows leukocytosis, neutrophilia and eosinophilia. The symptoms listed, in the absence of any signs characteristic of influenza, make

the diagnosis of scarlet fever.

Brucellosis. The early diagnosis of the disease on the basis of the clinical symptoms (fever, headache, depression, neural, bone, muscle and joint pains, perspiration, relative brachycardia and hypotension, hyperemia of the mucous membranes of the upper respiratory tract and throat, leukopenia, lymphocytosis and monocytosis in the blood) offers difficulties as a result of the similarity of brucellosis with influenza. However, the specific epidemiological history, the gradual development of the disease accompanied by enlargement of the liver and spleen, sometimes by a rash, lymphadenitis and fibrocytis speak more for brucellosis than for influenza. The following laboratory data assist in making the diagnosis: the Burnet test, the serological test of Wright and Huddleston, the opsono-phagocytic reaction of leukocytes of brucellosis patients with respect to microbes of the brucella group (Bilibin, 1950). In a number of cases the establishment of an undulating fever characteristic of brucellosis and a positive blood culture are too late to assist the physician in the diagnosis of influenza.

Subacute Bacterial Endocarditis. The onset of a case of subacute bacterial endocarditis is sometimes taken for the severe form of influenza, which is not accompanied by any pronounced catarrhal signs. The external appearance of the patient -- the pale-yellow-bluish color of the face, the dry lips, the luster of the eyes, and the hot cheeks are not characteristic of an influenza patient, nor is the hectic-type fever, which is accompanied by a chill, skin eruptions, enlargement of the liver and spleen and lability of the pulse. The signs of endo- and myocarditis, arthralgias, changes in the kidneys (focal nephritis) the rapid onset of anemia against the background of a prominent blood picture (at the beginning of the disease there is usually leukocytosis, neutrophilia, lymphocytopenia, monocytopenia, marked shift to the left, accelerated sedimentation rate) speak for the duration and seriousness of the disease. A positivie blood culture makes it possible to differentiate one disease from the other.

Pappataci Fever. Beginning with the first few days of its occurrence Pappataci fever resembles influenza: a sudden onset without prodromata, chill, rapid temperature rise, headache, muscle pains, hyperemia of the face, sclerae, conjunctivae, adynamia, tenderness on pressure of the eyeballs and on movement of them, leukopenia and relative lymphocytosis with a shift to the left. However, the disease is encountered only in a locality where the Phlebotomus papatasii lives during the season of hatching of the phlebotomus -- in July-August. Of the clinical symptoms which dis-

tinguish pappataci fever from influenza mention should be made of the following: 1) Pick's sign (injection of the scleral vessels in the form of a triangle facing with its apex toward the cornea and its base toward the outer angle of the eye); 2) Taussig's sign (pain on slight raising of the upper lid); 3) absence of catarrhal signs; 4) tachycardia at the climax of the disease; 5) marked bradycardia with fall in temperature; 6) normal blood pressure, decreasing somewhat during recovery. All these signs aid in distinguishing pappataci fever from influenza.

Tularemia. A sudden acute onset -- chill, temperature rise, depression, headache, pains in the muscles, scleritis, and cough -- may be the cause of confusion between tularemia and influenza (Gauzner and Belitser, 1928). In the epidemic foci a disease among those who hunt water rats gives rise to suspicion of tularemia. In the glandular form, tenderness and enlargement of regional lymph nodes occurs on the second or third day after the onset of the disease. In the visceral form a general intoxication is noted, enlargement of the spleen, skin eruptions. After an aspiration infection the pulmonary form of tularemia may develop. The following are used as special laboratory tests: 1) the allergic intracutaneous test with tularin, which with a positive result produces an infiltrate with hyperemia and edema beginning with the third to sixth day of the disease; 2) agglutination reaction with tularemic diagnosticum in a titer of no less than 1:100 (this reaction is of retrospective significance, because agglutinins appear in the blood of the patient only 10-15 days after the onset of the disease); 3) the biological method, that is, infection of a guinea pig with blood or with a smear taken from a lymph node of a tularemia patient or a culture of these materials. Therefore, the existence of characteristic clinical signs and valuable laboratory methods for the diagnosis of the disease aid in a recognition of tularemia.

Infectious Mononucleosis. (Glandular fever, Filatov's disease). Catarrhal signs in the nasopharynx and throat as well as the toxic state give the basis for mistaking infectious mononucleosis for influenza during the first few days of the disease. The occurrence of tonsillitis, enlargement of the lymph nodes (particularly the posterior cervical) as well as the enlargement of the liver and spleen along with hemorrhagic signs (bleeding from the nose, petechial rash) speak against influenza. A leukocytosis, neutropenia, monocytosis and atypical lymphocytes are noted in the blood. A positive Paul-Bunnel reaction confirms the diagnosis of infectious mononucleosis.

Hemorrhagic Fever. The epidemiological characteristics of the disease should be kept in mind in diagnosing

hemorrhagic fevers (Crimean, Omak): people become sick in the summer months (July-August) in localities inhabited by ticks -- the vectors of the infection. Apart from the general signs the following are most characteristic: marked hyperemia of the oral mucosa, enanthem in the throat, dry skin with a petechial eruption, hemorrhages as well as an enlargement of the lymph nodes, sometimes of the liver and spleen, and cerebral symptoms. The blood shows a small number of monocytes and the presence of Türk cells. All this speaks for hemorrhagic fever as against influenza.

Hemorrhagic Nephroso-Nephritis. Like influenza the disease begins acutely -- with a rise in temperature accompanied by headaches, hyperemia and puffiness of the face.

The main distinction from influenza is a considerable hyperemia of the oral mucosa with small hemorrhages as well as an erythema of the skin, face, neck, back and petechial eruption. In addition, a pronounced hyperesthesia of the skin, fibrillar twitching of the muscles and inconstant meningeal signs are noted. The disease is characterized by hemorrhages from various organs (nose, kidneys, sometimes lungs, stomach and intestine) and by hypotension. The blood shows leukopenia which changes into a leukocytosis with a marked shift to the left and Türk cells.

Malaria. The clinical aspects of malaria are characterized by a cyclic alternation of paroxysms accompanied by chill, with a subsequent sharp rise in temperature and profuse perspiration. Before the onset of prominent symptoms it is sometimes difficult to distinguish the first attack of malaria from influenza during the first two or three days of the disease. However, the facial pallor at the beginning of the disease, the marked chill, sometimes shaking, an enlargement of the spleen, the occurrence of remissions which alternate with the paroxysms, indicate malaria. At the very beginning of the disease a blood analysis (thick drop) with the detection of the malaria plasmodia aid in clarifying the diagnosis.

Swamp Fever. In those areas where cases of swamp fever (non-icteric leptospirosis) are recorded this disease is frequently confused with influenza on account of the related nature of the symptoms at the beginning of the disease.

For the purpose of clarifying the diagnosis the epidemiological history is of importance: swamp fever is observed in the summer time, during the hay-making and bathing period. In swamp fever the spleen and liver are enlarged, and sometimes there is a rash. There is leukocytosis in the blood. The diagnosis is confirmed by the following laboratory examination: the finding of leptospiras in the blood, a positive blood culture, and the agglutination reaction (patient's blood serum plus leptospira culture).

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Relapsing Fever. Relapsing fever is distinguished from influenza by the following symptoms: marked chill, tachycardia, pains in the gastrocnemius muscles. The external appearance of the patient (pallor), the rapidly enlarging spleen and liver, the absence of catarrhal signs and leukocytosis are not characteristic of influenza. The diagnosis is confirmed by finding the Obermeier spirilla in a thick drop of blood. This problem has lost its importance at present because of the fact that relapsing fever has been eliminated in the Soviet Union; however, it may be of importance in localities where there are natural foci of tick-borne relapsing fever.

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NINTH CHAPTER

TREATMENT

81. Experimental Therapy of Influenzal Infection

The experimental therapy of influenza developed with the study of the etiology, pathogenesis and immunity of this disease. Since all these problems began to be studied on a solid scientific foundation after the discovery of the influenza pathogens, the experimental therapy of influenza is a new division in the study of this infection. The main divisions, which have been developed in the past 10-15 years, are, first, the search for chemotherapeutic substances which might suppress the development of the influenza virus in the body (chemotherapy of virus influenzal infection) and the bacterial microflora (chemotherapy of secondary bacterial infection), and, secondly, the development of methods of immunotherapy.

The development of the chemotherapy of virus influenza has been the most difficult problem. This was associated with the general difficulties in solving this problem. Definite achievements which have been made in the last two decades in the field of chemotherapy of bacterial infections stimulated investigators to use sulfonamides and antibiotics for the chemotherapy of virus infections. As it is well known, these very active preparations turned out to be effective only for large viruses (rickettsias and chlamydozoans) and completely inactive with respect to the other viruses, including influenza viruses. For some time it has been thought that everything depends on the success of finding a suitable antibiotic or chemotherapeutic agent. Therefore, tests were made of various chemical preparations, activity products of fungi and bacteria, as well as other biologicals. However, the very modest results caused many investigators to proceed along another route -- the study of the mechanism of virus multiplication in the body and a study of its metabolism in order to find methods of influencing the virus and suppressing its development. At the present time, both these trends are represented in the

investigations of the chemotherapy of influenza.

The study of the chemotherapeutic effect of various substances on influenza viruses is made usually on mice, chick embryos and in tissue culture. There is no point in presenting the methods used for this kind of investigation here, although they have their own essential characteristic features in certain detail (Dreyzin, 1954).

Usually, the criterion for evaluating the activity of various preparations in the quantity and dynamics of virus accumulation in tissue cultures, in a deembryonated egg, chick embryo, or mouse lungs or the therapeutic effect which is manifested in a reduction in the death rate of animals and a decrease in the severity of the pathology.

All the sulfonamides tested up to the present time have proved to be without any effect on the influenza viruses (Harford and others, 1946; Cogeshall and Mayer, 1942; Sokolov, 1943).

The antibiotics, most active against bacteria, have also proved to be ineffective (streptomycin does not affect the multiplication of influenza viruses in chick embryos even in large doses (Florman and others, 1946)). The other well-known antibiotics -- penicillin, chloromycetin (synthomycin, levomycetin), aureomycin (biomycin), terramycin, gramicidin (Kass and others, 1950; Yermol'yeva and other, 1950; Dzagurov, 1953) -- have completely no effect on the influenza virus or else exert a weak effect, for example biomycin (Dzagurov, 1953).

Phytoncides of onion and garlic, which after prolonged use have provoked a latent infection in mice (Yakovlev and Zvyagin, 1950), have also proved to be inactive. A certain activity in vitro of part of the preparations tested (penicillin, aureomycin and terramycin), which was shown when they were used in large doses (Yermol'yeva and others, 1950; Dzagurov, 1953), is apparently explained by an effect similar to that of disinfectants, which could certainly not be manifested in the body.

According to the data of Pershin and co-authors (1954), dextromycetin possesses a chemotherapeutic effect in experimental influenzal infection in mice, but the checking investigations of Dzagurov (1953) have not confirmed these data.

Lysozyme does not exert any depressive effect either on the influenza virus or on the erythrocyte receptors (Colucci and Jacono, 1950).

Hirst and Stacey (1950) tried out the effect of 160 different bacterial cultures on the influenza virus as well as the effect of bacterial and other polysaccharides. They all proved to be inactive, and only the injection of certain macromolecular substances four hours before the infection

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(gluten and carboxymethylester-amylase) mechanically protected the mice for a certain time against the influenza virus.

Tannic acids (Carson and Frisch, 1953) possess a certain inactivating effect through the binding of free virus outside the cells.

In Green's experiments (1949) tea extracts considerably inhibited the development of the influenza virus.

Of other substances the acridines have attracted particular attention; many of them actively inhibit the influenza virus multiplication (Rasmussen and others, 1947; Rasmussen and Stokes, 1951; Zeytlenok and Vagzhanova, 1953).

In the experiments of Takemoto and others (1954) sulfonic acid benzoide, Congo red, formicin and certain other compounds possess a multiplication-inhibiting effect, but in experiments on mice they all proved to be ineffective.

A series of investigations directed at looking for chemotherapeutic agents for influenza was carried out by Dreyzin (1954). In the investigation of 151 specimens of actinomyces culture fluids obtained from the laboratory of Krasil'nikov, several relatively active series were selected. However, it was impossible to increase their activity by chemical fractionation. More active and stable preparations were obtained by investigating extracts from leaves of 19 species of eucalyptus and 10 species of myrtus (Dreyzin and Sokolova, 1954). Study of the nature of the active principles made it possible for the authors to classify them among the tannates, which being adsorbed onto the cells prevent the penetration of the virus into them. On subsequent investigations, the authors (Dreyzin and Karlina) obtained two other active preparations -- an iodine preparation and a commercial apple pectin. The active principle of the latter was not associated with the pectins.

In recent years, works have been published concerning the effectiveness of a new antibiotic in experimental influenzal infection, ehrlichin (group E and others, 1951). The preparation was shown to be effective also in other virus diseases.

Preparations of ecmoline have proved to be more active; these were suggested by Yermol'yeva, Shubladze and Gaydamovich (1950) and represent antibiotics of animal origin obtained from the milt of certain species of fish. Yermol'yeva, Shubladze and others (1952, 1953) performed experiments on mice, showing the presence of a definite therapeutic effect of this preparation in experimental influenzal infection.

Whereas in a control group all mice became sick with severe confluent influenzal pneumonia, the lungs were unaffected in more than one-third of the mice which had been

given the influenza virus with ecmoline. In the other mice a focal bronchopneumonia was observed, and a typical influenzal pneumonia was noted only in isolated animals. In the group of mice which had received the Shklyaver strain of influenza virus and ecmoline, unaffected lungs were found more often than in mice which had been given the PR8 virus and ecmoline.

In the experiments of Dzagurov (1953), ecmoline exerted a distinct inhibiting effect on the development of the influenza virus in chick embryos; its effect on the influenza infection of mice was somewhat weaker.

These data have served as the grounds for testing the preparation clinically.

Numerous investigations have been developed recently on the study of the effect of various inhibitors of multiplication of influenza viruses and other viruses. These investigations, in elucidating the characteristic features of virus metabolism, have at the same time opened up routes for scientific development of chemotherapy of virus infections (Ryzhkov, 1953).

In the experiments of Tovarnitskiy, Shubladze and Gaydamovich (1950) a part of the detergents studied possessed a certain activity.

According to the data of Hennen (1950), α-tocopherol esters inhibit the enzymic activity of the influenza virus in vitro (in ovomucin) and protect chick embryos against small doses of the virus. Proflavine proved to be active with respect to the B virus but not the A virus (Briody and Stannard, 1951). Complex synthetic polypeptides possess an inhibiting effect (Rubin and others, 1951). The adrenocorticotropic hormone proved to be inactive in influenzal infection (Loosli and others, 1951; Kass and others, 1951).

Zeytlenok and Vagzhanova (1953) studied the effect of 22 substances on the development of the influenza virus in chick embryos. Of the acridine group stabrine and proflavine inhibited the development of the influenza virus; of the thiosemicarbazone group, the preparation cutisone [thiosemicarbazone-p-isopropylbenzaldehyde]; from the amino acid group, histidine; and from the dicarboxyl acid group, malic acid inhibited the influenza virus development. According to the data of Lutikova (1953, 1954), in experiments on deembryonated eggs the amino acids norleucine and glutamic acid distinctly suppressed the multiplication of the virus; cysteine, inconstantly; and in the dicarboxyl acid group, malic acid.

A suppressive effect on the influenza virus multiplication is shown by 2,4-dinitrophenol (Ingraham and others, 1953; Eaton and Perry, 1953), aromatic diamidines -- pentamidine and stilbamidine (Eaton and others, 1952;

McClelland and van Rooyen, 1949), sodium monofluoracetate (Mogabgab and Horsfall, 1952), triphenylmethane (Hoyle, 1949), sulfonic acids (Ackerman and Massab, 1954), α-amino-sulfonic acid (Ackerman, 1952), chlorine derivatives and other benzimidazole (Tamm and others, 1953, 1954) derivatives.

In the experiments of Vorob'yeva (1954) an inhibitory effect was shown on the multiplication of the influenzal virus by substances which interfere with the synthesis of adenosinetriphosphoric acid during the processes of glycolysis (sodium fluoride), substances inhibiting the formation of adenosinetriphosphoric acid by oxidative decomposition (malic acid) and substances which separate the processes of respiration and phosphorylation (dinitrophenol).

Despite the considerable volume of investigations which have been made, no highly active chemotherapeutic agents have been found yet for the influenza virus. Of the preparations which show a more or less pronounced activity ecmoline, stabrine and cutisone have been applied clinically.

The chemotherapy of the secondary bacterial infection in influenza has been based on numerous studies which have established the spectrum of action of the most widespread antibiotics and chemotherapeutic agents. Therefore, few experiments have been required to show the applicability of chemotherapy of bacterial infections to influenza.

The effect of sulfonamides on infection produced by the association of the influenza virus with the pneumococcus and streptococcus has been studied by Sokolov (1943) and Harford and co-authors (1946). In the experiments of these authors it was shown that sulfonamides inhibit the development of a pneumococcal infection both in itself as well as in association with the influenzal infection, without affecting the latter. Thereby, it was clarified that sulfonamides when used by inhalation in doses of 200 times less than the oral doses exerted the same effect (Sokolov, 1948).

The influence of antibiotics and microbes encountered in influenza has been studied by Dzagurov (1953). Pneumococci and streptococci proved to be sensitive to synthomycin, levomycetin, aureomycin, biomycin, penicillin and ecmoline. Dextromycetin did not influence the growth of the pneumococcus and streptococcus.

Therefore, in experiments the expediency has been proved of using antibiotics and sulfonamides in influenza for suppression of the bacterial microflora as a method of preventing the development of secondary infections and complications.

As far as immunotherapy is concerned, this trend has been realized in the form of application of an anti-influenzal specific immune serum. This serum, which was first

extensively studied by Smorodintsev and Shishkina (1938), was afterwards perfected (Orlova and Kalinina, 1954). At the present time, this preparation is being released in the form of a powder containing immune serum, sulfonamides and penicillin, and is used by means of blowing it into the nose by means of a special atomizer.

82. General and Nonspecific Methods of Treating Influenza

For the purpose of treatment and prevention of influenzal diseases a tremendous number of all-possible agents, measures and methods have been proposed. For example, bleeding, stimulants, emetics, purgatives, antispasmodics, etc., have been used. At the present time, this is only of historic interest. We should like to mention only a number of measures which have been used in the past decade for the purpose of preventing and treating influenza and its complications, chiefly pneumonia.

Most frequently, salicylates have been used, which have been prescribed at times for their antipyretic and at times for their analgesic effects. The most popular of them are aspirin and antipyrine salicylate, which were hardly considered specific. Sodium salicylate, antipyrine phenacetin, pyramidone and others were also prescribed, sometimes in all-possible combinations or with the addition of caffeine, camphor, codeine, cincophen and others (Volkovinskiy, 1936; Kogan, 1936; Mailyan, 1941, and others). Many persons recommended the combination of salicylates with other preparations, for example cilotropin (sodium salicylate plus urotropin plus caffeine salicylate) or salitropin [salicylate plus atropine] (Kirstner and Gorinshteyn, 1933; Mashkov, 1938, and others), pyramidone with urotropine (Rossiyskiy, 1937, 1938), and various other combinations (with codeine, cincophen, etc.). Quinine was suggested (Strauss, 1937; Spitta, 1933) in various doses (by mouth and parenterally) and various derivatives of it (optochin, quinoline, chinosol, etc.), as well as combining them with other preparations, for example, with calcium, salicylates (Gmeiner, 1929; Wreschner, 1929; Scherf, 1936). Others have denied the efficacy of quinine (Gubinskiy (1937), Zeytlenok (1940), Fasching (1943), and others), indicating the harmful influence of optochin and certain other quinine derivatives on vision. Rubashova (1935), Braude (1939) and Epshteyn (1944) did not observe any effect from antipyretics.

Urotropine (by mouth and intravenously) and calcium chloride have been used extensively, particularly the combination of them -- calcex, which has been recommended by a number of authors as well as by the Commission on Influenza of the Scientific Medical Council of the Ministry of

Health RSFSR. Their effect is regarded as antiseptic as well as one which tonicizes the vegetative nervous system (Ionin, 1947; Antipin, 1936; Kogan, Biskin, 1927; Rossiyskiy, 1940). Other authors do not confirm the efficacy of these agents (Rubashova, 1935; Sandler, 1941; Epshteyn).

A number of authors point out the efficacy of small doses of iodine (Agroskin, 1935; Senderikhin, 1937; Vorob'yev, Zhdanov and others, 1942), but Sandler (1941) does not confirm its efficacy.

Once, many persons were attracted by another halide, chlorine, and spoke out for its great efficacy. There is an extensive literature on the problem of using chlorine in the treatment of influenza (Somoylov, 1930; Lukov, 1931; Tsitovich, 1931; Fel'dshteyn, 1932; Yegorov and Naranovich, 1934; Yurchenko, 1934; Yudin, 1934; Yastrebova, 1935; Bondarenko, 1935; Kovnatskiy, 1936; Bagdasar'yan, 1937; Kalina, 1938, and others). However, many authors have not been able to confirm the efficacy of chlorine in animals (Smorodintsev and Shishkina, 1937) or on people (Bliokh, 1930; Shteynshneyder and Yakubson, 1931; Sokolov, 1932; Kasumov, 1936, 1940; Gurovich, 1938; Ansheles, 1934, and others). The bactericidal effect of chlorine inhalations on the microflora of the upper respiratory tract, which many authors associated with a positive therapeutic and prophylactic effect of chlorine in influenza, is denied by other authors (Kreshchenko, 1934; Stepanov-Grigor'yev and others, 1937; Smorodintsev and Shishkina, 1937). The safety of using chlorine in a low concentration -- 0.015 gram of chlorine per liter of air -- has been confirmed in the literature (Rozenberg, 1934; Chilikin and Nabokov, 1927).

There are reports concerning the efficacy of treating influenza and its complications with collargol [antiseptic silver preparation] (Wachter, 1918; Bannikova, 1926; Dmitriyev, 1937), colloidal silver albumin tannate (Korbsch, 1934), ichthyol (Vaynshtok, 1927; Toskin, 1927), trypaflavine (Crohn, 1920; Zacharias, 1928; Rozenberg, 1934), arsenic preparations, salvarsan (Shemenskiy, 1919), menthol in oil by mouth (Schapec, 1933), aconite (Erdmann, 1934), pleon (Zeyfart, 1953), togal (Gross, 1926), pyridium (Lorenzen, 1937), strychnine (Bonnin, 1929), milk intramuscularly (Paczkowzky, 1919), thyroid iodine (Shevelev, 1936), pilocarpine (Monnero-Dumaine, 1932), atropine (Wheelarand, 1944), omnadin (Friedman, 1927), and formic acid (Kasumov, 1940).

For the therapy of influenza use has been made of lysates (Slavin, 1936), autohemotherapy (Yakhontov, 1931; Glazko and Gornakov, 1932; Zhukovskiy, 1937), blood transfusion, autogenous serum, convalescent serum (Béclère, 1938; Torrens, 1934), sera containing antibodies against the supposed pathogen -- pneumo-, streptococci, Bacillus influenzae

and others (Spengler, 1919) with various degrees of success as well as diphtheria antiserum (Crohn, 1920).

A number of authors have noted the great therapeutic and prophylactic efficacy of various physiotherapeutic procedures: compresses, poultices, including mustard-plasters (Veysfel'd and Kruts, 1941), baths for the whole body and for the feet, diathermy (Yakovlevskaya, 1937) roentgentherapy (Yal'tsev and Potekhin, 1937), radiant energy (Basov and Tsaturova, 1935; Fridlyand and Bessonova, 1935), irradiation with ultra-violet rays (Dolgikh, 1937; Mar'yasin and others, 1938; Vitebskiy, 1940; Brusnikin, 1950), sollux, electroaeroionization, particularly in combination with quartz (Vilenkin, 1931, 1935; Rossiyskiy, 1937, 1949; Koroleva and Smusina, 1952) and dionin-iontophoresis (Trotskaya, 1951).

There is a review of the popular medicines used for the treatment of influenza in the works of Gryaznov (1949, 1951).

Everything which has been stated above is evidence to the effect that we do no have any definitely reliable measure against influenza.

Depending on the nature of the material which has come under the observation of various observers different conclusions are obtained concerning the efficacy of the method of treatment recommended.

Influenza and acute catarrhs of the respiratory tract which are frequently confused with it represent different diseases with different etiologies, epidemiologies, pathogeneses and clinical aspects. The differential diagnosis of these diseases and a selection of the appropriate therapy are necessary.

By virtue of the contagiousness of the disease the patient with influenza needs to be isolated. If there is a separate room, the patient should be placed there; however, such a possibility does not always exist if the family lives in a single room. Then an attempt must be made to isolate the patient as much as possible within the limits of the same room. It is necessary to explain to those around that the patient should sleep by himself, that he should be put into a corner of the room apart from everybody else and, if possible, his bed should be partitioned off (with a curtain, sheets, etc.). Using these measures the sprays of mucus and saliva which come from speaking, coughing and sneezing will be scattered around the room to a lesser degree. The patient should make use of a separate towel and handkerchief. On sneezing and coughing he should cover his nose and mouth with his handkerchief in order not to spray the room with saliva, mucus and sputum. A cuspidor should be placed near the patient,

and it is desirable that some kind of disinfectant solution be poured into it, for example, two percent chloramine solution, lysol, etc.

The patient should observe a bed-rest routine. Even if he feels relatively good he should lie down both in his own interests, in order to get better more quickly and to prevent the possible complications, and for the purpose of disseminating the infection to a lesser degree.

The patient should be wrapped up warmly. It is recommended that he be given hot tea, milk, tea with raspberries, and linden flowers; the hot drink increases perspiration and diuresis, and therefore contributes to the removal of toxic substances from the body. In addition, by moistening the mucous membrane of the mouth, throat and pharynx the drink softens it and mollifies the inflammatory process.

As a rule, the patient with influenza does not require a special diet. The usual diet for the febrile patient is recommended. If the patient's appetite is maintained he can eat everything. Nevertheless, he should be advised to avoid dishes which cause an irritation of the mucous membranes of the respiratory tract. These are sharp, acid products, for example, pepper, mustard, vinegar, horse radish, as well as nuts, etc., which irritate the mucous membranes of the respiratory tract and increase the cough.

The room where the patient is should be aired as frequently as possible, because this way the room air is removed in which the very tiny droplets of saliva and mucus containing the influenza virus excreted by the patient during conversation and coughing are floating. It is better if the room is on the sunny side and is illuminated by the sun; under these conditions, the room and the patient himself are disinfected and exposed to the salutary effect of the sun's rays.

The cleaning of the room should be accomplished by the wet method. It is useful to rub wooden objects (floor, door) with 0.5 percent chloride of lime solution or two percent chloramine.

In taking care of the patient and after contact with his body, underwear, or bed the hands should be washed carefully.

In treating influenza it is recommended that symptomatic measures be used in the presence of the appropriate indications. These are cupping, mustard-plasters, gargling, expectorants, narcotics for dulling the cough, antineuralgic agents, etc.

From the very beginning of the disease the patient's cardiovascular system should be watched (collapse is possible), and where necessary various agents should be used

(camphor, caffeine, nikethamide, adrenalin, glucose infusion and infusion of physiological solution).

Certain pathological signs sometimes require the use of special measures. Thus, for example, sharp headaches, muscle aches or nerve pains are relieved by taking pyramidon two or three times a day, phenacetin, aspirin and other analgesics, which are good to use in combination with caffeine. With excitation or insomnia bromides should be given in various dosages, as should also Bechterev's mixture. In cases of marked apathy, depression, or drowsiness where there are no contraindications (old age, arteriosclerosis, hypertension) amphetamine in small doses may be indicated. When there is a dry painful cough, cupping, mustard-plasters, codeine, dionine, heroin or Dover's powder should be prescribed three times a day. When there is a productive cough, and the sputum does not come up well, expectorants are prescribed: alkalis (for example, hot milk in a 50:50 proportion with borjom [mineral water found in the Borzhom region in Georgia] or with the addition of half a teaspoonful of drinking soda to a glass of milk (which is taken in a dose of half a glass several times a day), ipecac root, thermopsis, senega root with the addition of alkalis, for example, amonium chloride-anise drops, sodium benzoate, sodium carbonate. When there are pathological signs in other organs and systems (digestive tract, liver, urinary tract, etc.), the patients need the use of various agents depending on the pathological signs observed. The sympathomimetic agents (adrenalin, ephedrin) should be kept in mind, as should also agents which depress the parasympathetic functions (atropine, belladonna) because not uncommonly a considerable predominance of parasympathetic functions of the vegetative nervous system is observed over the sympathetic functions, and a depression of the latter. Other agents should not be forgotten if the corresponding indications for them exist. For example, when there is dryness in the throat gargling should be prescribed, and hot beverages should be given to drink; when there is dryness in the nose, vaseline, menthol drops, protargol, etc., should be prescribed. In short, the symptomatic pathogenetic agents should not be ignored which improve the feeling of well being and the general condition of the patient.

83. The Use of Antibiotics and Chemotherapeutic Preparations

Of this group of preparations ecmoline, atabrine and cutisone (which act on the influenza virus), sulfonamides and bacterial antibiotics (which act on the secondary bacterial infection and influenza) have attracted attention.

In recent years, Yermol'yeva's preparation, ecmoline, has been included in the practice of treating influenza. This antibiotic of animal origin has proved to be effective when tested in the laboratory on mice (Shubladze) and chick embryos (Dzagurov).

Ritova (1951, 1953) has used it successfully for the treatment of influenza in children. The preparation was injected intranasally three times a day in children under one year of age during the first three days of the disease and a dose of 0.25 cubic centimeter for each injection; it was given to older children in a dose of 0.5 cubic centimeter.

The fever in those treated with ecmoline was less pronounced, and the signs of toxic state disappeared sooner.

According to the data of Yermol'yeva and co-authors (1953), the use of ecmoline in the form of drops intranasally as well as the inhalation of it in the form of an aerosol or sucking the tablets of this preparation shortened the duration of the fever and of the disease and led to a reduction in the number of complications (Table 110).

Table 110

Efficacy of Ecmoline Treatment

Preparation	Average duration in days		% of complications
	fever	disease	
Virus Influenza			
Ecmoline	1,8	5,2—5,6	0—2
Sulfonamides and pyramidon without ecmoline	2,9	6,3	6—10
Ecmoline + penicillin	1,9	4,8	0—2
Sulfonamides and pyramidon without ecmoline	3,2	6,4	8
Acute Catarrhs of the Upper Respiratory Tract			
Ecmoline + penicillin		3,2	0—1
Without ecmoline		4,8	8

In severe cases of influenza complicated by pneumonia the authors recommend using ecmoline with penicillin intramuscularly twice a day or ecmonovocillin intramuscularly once a day.

Khersonskaya (1953) also obtained beneficial results in the treatment of influenza patients with ecmoline (1953). In patients the duration of the fever decreased somewhat, and the degree of intoxication was reduced (Table 111).

Table 111

Results of Treatment of Influenza With Ecmoline

Treatment	Average duration in days			
	fever		intoxication	
	mild form	moderate form	mild form	Moderate form
With the use of ecmoline	3,3	4,2	5,0	5,6
Without the use of ecmoline	4,5	5,2	5,6	6,8

However, the frequency and character of the complications in those treated with ecmoline were unchanged in comparison with those in patients treated by the usual measures.

Loban (1954) also obtained favorable results in the use of ecmoline with penicillin.

Ecmoline, which we used in the hospital in 1949 in the form of intramuscular injections in a dose of one cubic centimeter (two cubic centimeters produced a transitory pain at the site of injection) three times a day or in the form of instillations (three times a day in a dose of six to eight drops into each nostril) and even better in the form of inhalations in a dose of one cubic centimeter two or three times a day, somewhat shortened the duration of the fever and of the disease. In this dosage the preparation did not cause any side-effects. Therefore, ecmoline can be recommended for observation in general practice (Shimshelevich and Fadeyeva, 1952; Epshteyn and others, 1952; Ritova, 1951; Khersonskaya, 1953, and others). With the addition of penicillin the efficacy of the preparation is increased on account of the effect on the microflora --

the accompaniment of influenza. This has an effect in decreasing the number of complications produced by the coccal flora, which ecmoline itself does not prevent. In addition, ecmoline possesses a prolonging and syngergistic effect when it is used simultaneously with penicillin (Yermol'yeva). We cannot give any final evaluation of the efficacy of ecmoline because of the insufficient number of patients who have been under observation.

In 1950, a better result was obtained after the intranasal administration of ecmoline than after its parenteral application. Ecmoline was most effective during an outbreak of influenza in 1949. The preparation has not been standardized, and it is possible that this explains its different degrees of effectiveness in different epidemics. It is expedient to inject it intranasally (better by inhalation in the form of an aerosol) in combination with penicillin.

Of the chemotherapeutic agents atabrine deserves attention. Acridine preparations (for example, rivanol, trypaflavine, atabrine) proved to be inhibitors in experiments on embryos. They checked the development of viruses, including also the virus of type A and B influenza.

After making sure in the hospital that the influenza patients tolerate atabrine well, we shifted our observations to a military infirmary, where Dr. Panasyuk, under our supervision, treated patients with influenza caused by different types of the virus. Atabrine was used in a dose of 0.3 gram a day until the temperature dropped and for another day, when the temperature was normal. It was given for two and rarely for three days. On the first day, it was recommended that the entire 24-hour dose be given at one time, and beginning with the second day that a shift be made to a dose of 0.1 gram three times a day. The contraindications to the use of this preparation were the same as for malaria. The results proved to be satisfactory, particularly when the treatment was begun on the first day of the disease. Beginning with the second day, the results obtained were not bad either, but beginning with the third day no particular effect was obtained. This is apparently explained by the fact that the atabrine acts on the influenza virus. If it is given at the very beginning of the disease it exerts an effect which inhibits the influence of the virus on the body. If it is begun on the third day of the disease, when the virus is becoming more active and other forces are entering into the effect, particularly microflora on which the atabrine has no effect, use of the preparation comes to naught.

Our observations on the effect of atabrine on influenza were made on 83 patients under hospital conditions

during influenza outbreaks in 1949 and 1950. This number of patients was insufficient for arriving at any final conclusions, but the results obtained were very encouraging.

According to our reports, Ashbel' began to use atabrine treatment of influenza patients during the outbreak of 1952. He injected it in the form of an aerosol in a dose of 0.1 gram of atabrine per inhalation, starting the treatment at the very beginning of the disease after detecting the patients by means of prophylactic examinations at their place of work. Only two patients of the 81 were put on the sick lists. In the others, the temperature became normal on the same day. Sorokina (1956) notes a reduction in the fever by one day and of the disease by 1.3 days after atabrine treatment (104 patients).

Atabrine proved to be effective when it was used on influenza patients (more than 400 persons) under hospital conditions and polyclinic conditions (Semashko, 1950; Epshteyn and Panasyuk, 1951; Berlyant and Epshteyn, 1953; Ashbel', 1954). A reduction in the febrile period and in the duration of the disease was noted in comparison with the control group of patients who were given antipyretics, sulfonamides and ecmoline.

In this connection, the observations on the prophylactic effect of atabrine in influenza were interesting. According to the data of Panasyuk, in one military unit where he used atabrine for the prophylaxis of influenza according to the method which has been adopted for the prophylaxis of malaria relapses, the influenza morbidity rate was three and a half times less than in the control military unit where the prophylaxis was accomplished with urotropine. Ashbel' made the following observations during an influenza outbreak in 1952. In one shop, 108 workers were given 0.1 gram of atabrine each prophylactically, which was dissolved in two cubic centimeters of water by means of inhalation in the form of an aerosol once in seven to nine days, in a total of three times for the entire period of the outbreak (90 workers from this shop served as controls). Of the 108 workers who had been given atabrine one became sick with influenza, and of 90 workers in the control group, 14 became sick.

Rybakov (1954) who used atabrine in the form of an aerosol in 350 persons, as well as Gal'per (1953), who prescribed the preparation by mouth two days straight in a dose of 0.2 gram at intervals of five days (1475 persons), gave good reports concerning the prophylactic effect of atabrine in influenza.

We should like to discuss the preparation cutisone, which has been synthesized at the All-Union Scientific Research Pharmaceutical Institute. Cutisone belongs to the

group of thiosemicarbazones. In experiments on embryos it proved to be an inhibitor which checked the development of influenza viruses (Zeytlenok and Vagzhanova). For the purpose of treating influenza it was used with good results by Filippova-Nutrikhina (1954). On testing both under clinical and polyclinic conditions cutisone proved to be more effective than other agents used as controls (sulfonamides, antipyretics) in connection with reducing the temperature reaction (from 0.5 to one day or more) and the disappearance of the other signs of a toxic state. It lagged somewhat in its efficacy behind influenza antiserum (according to the data of Leshchinskay and Semashko, 1954); the toxic signs remained longer than after treatment with serum. Cutisone is very bitter. It is released in small tablets containing 0.005 gram and is prescribed by mouth in a dose of one tablet three times a day for an adult (for children the dose is reduced correspondingly). In this dosage the preparation does not produce any side-effects.

Of the other chemotherapeutic agents against influenza the sulfonamides have become most widespread, although they do not affect the influenza virus. The sulfonamides are given by mouth and are instilled intranasally.

Certain authors have recommended sulfanilamide (Ionin, 1942, and others). We have not noted any particular effectiveness of the preparation in treating influenza or colds. Rossiyskiy (1946) observed favorable results in the use of sulfanilamide in combination with urotropine. Other sulfonamide preparations were tested also with varying degrees of success (Shub, 1944; Epshteyn and others, 1948).

In treating influenza with sulfapyridine in combination with sulfanilamide, we, as well as Semashko, Bibikova and others, have not observed any particular effect. Shub has successfully treated influenza by spraying one gram of sulfapyridine into the patient's nose every day. By this method of treatment Semashko observed fewer complications.

Adamson and Flatt (1942) point to the absence of any therapeutic effect in sulfanilamide therapy of influenza.

In the treatment of influenza sulfonamides have been used also in the form of the silver salt of sulfathiazole (Filatov, 1949, 1953) and a triple combination of sulfonamide preparations: sulfapyridine, sulfamethylthiazole and sulfanilamide in equal doses (Shavrova, 1945).

We have not observed any particular effectiveness from the daily intranasal instillation of the triple sulfonamide powder in a quantity of one gram or after the injection of eight to ten drops of a two-percent suspension of

the silver salt of sulfathiazole into each nostril three times a day.

We studied the influence of sulfonamides on the influenzal infection during the outbreak of influenza in December in 1943-January 1944. Then, we gave four grams of sulfapyridine, sulfamethylthiazole or sulfadiazine and 1.2 grams of prontosil a day (one gram of sulfapyridine and 0.3 gram of the prontosil by mouth four times a day). In order to exert an effect on the microflora which developed in influenza and which produced complications, because we knew that the sulfonamides do not exert any effect on the influenza virus itself. We did not obtain any notable reduction in the febrile period, and could not find any definite reduction in the duration of the disease, but we had 6 1/2 times fewer complications than in the control (treating patients with urotropine).

Treatment of influenza patients with sulfonamides became abnormally widespread. The preparations began to be used without the appropriate indications and not in the proper dosage. This subsequently led to the carriage of sulfonamide-resistant races of microbes among the population, not to mention the fact that the body is not indifferent to sulfonamides because of their toxicity and side-effects, sometimes very serious. They should be prescribed in the presence or suspicion of influenza complications, for example, pneumonias as well as when the patient has a tendency towards these complications. The dosage per course of treatment should be no less than 23-24 grams, and the preparation should be given according to the system for treating pneumonia.

Sulfonamide treatment without appropriate indication does harm to the patient subsequently, not to mention that the body is not indifferent to the sulfonamides because of their toxicity and the very serious side-effects which are sometimes observed with their use.

Certain physicians have begun to use penicillin for the treatment of influenza, although it does not exert any direct effect on the influenza virus either. The preparation is injected intramuscularly and intranasally, and prescribed in the form of inhalations and by mouth. It is necessary to be concerned about the excessive increase in penicillin therapy as well as the disorderly application of antibiotics, because of the possibility of obtaining carriage of races of microbes resistant to these preparations.

Kofman (1948, 1953) and Ashbel' (1951), Salgo (1954), Gerasimenko and Savel'yeva (1953), Kurashova (1954), Smirnova (1954) recommend penicillin in influenza in the form of aerosol inhalations. For example, 100,000 units of

penicillin in two cubic centimeters of physiological solution twice a day or 200,000 units once a day. Kofman gave a one-day course of treatment with three inhalations every three hours using a dose of 30,000-35,000 units of penicillin in solution with a good result. This method of treatmenthas more sense than the parenteral metho and particularly the peroral method, because penicillin is being introduced where the microbial depot is located, and by this means a greater concentration of it is created there. In addition, it is very well absorbed from the surface of mucous membranes of the respiratory tract, and a therapeutic concentration is created in the blood which persists, according to Ashbel''s data, no less than a day after a single inhalation in the form of an aerosol containing 200,000 units of penicillin. The same thing is obtained if sulfonamides are introduced directly into the nostrils by means of spraying one gram of the preparation (sulfapyridine, sulfamethylthiazole or sulfamethazine). After a direct effect a greater concentration of the preparation is created in the respiratory tract than after parenteral use. The intranasal use of one gram of sulfapyridine once a day was tried out by Shub in Sverdlovsk, and in an influenza hospital, by Semashko. However, even after the application of penicillin by inhalation the question of penicillin-resistant races of microbes cannot be eliminated completely yet and awaits solution.

Of the other antibiotics, erythromycin has been used in influenza with a beneficial effect (Cronck and Naumann, 1954).

Certain authors indicate a favorable effect of treatment of influenza with garlic phytoncides (Kufman, 1951; Novikov, 1954), but these have proved to be ineffective in experiments on mice (Yakovleva and Zvyagina).

84. Serum Therapy and Other Methods of Immunotherapy

Vaccine therapy has not become very widespread in influenza, because the disease always has an acute course, is brief and is accompanied by a rapid rise in immunity.

In the past, bacterial vaccines were tested for the treatment of influenza, since at that time the virus etiology of influenza had not been proved. An unusual application of Bezredka's views was the use of an antivirus preparation suggested by Fal'kovich and Korobitsyn (1934).

Bacterial vaccines of a different kind were tried out -- mono-, poly- and autovaccines with variable results (Vyshegorodtseva, 1927; Gel'fgat, 1928; Sheboldayeva, 1934; Makarov, 1934; Steriopulo, 1936; Anderson, 1927).

Many authors have recommended the A antivirus for the

time, the serum did not exert any effect on many inflammatory processes, apparently of bacterial origin. In another work, Nechayev (1940) pointed out that when the serum is used early the percentage of pneumonic complications dropped to zero. The serum was effective for treatment in the first two days of the disease; when it was used later it was not very effective. Although Smorodintsev (1943) even at that suggested incorporating the serum on a broad scale into practice, the preparation needed to be improved, and this was accomplished only after ten years.

In 1939, we used this type of influenza treatment successfully. The method proved to be effective in the sense of reducing the fever, shortening the duration of the disease and decreasing the number of complications. It did not exert any particular effect on the catarrhal signs. In 1940, the use of the serum was unsuccessful because serum against influenza A could not be effective against influenza B, which was producing the outbreak in that year.

This method, however, is not generally available, because it cannot be used under domestic conditions. In addition, it was difficult to obtain the serum for treatment, and it required a complex apparatus.

Subsequently, Smorodintsev suggested a specific dry influenza antiserum which might be blown into the nostrils along with penicillin and sulfonamides by a powder-blower (Fig. 256) introduced into the nostril. The preparation is put into a small glass cylinder. On compression of the rubber bulb a stream of air takes up the powder, which penetrates into the nose, nasopharynx, and during inhalation, into the larynx, trachea and lower. The dry specific serum obtained from one cubic centimeter of blood serum, 10,000 units of penicillin and 0.5 gram of sulfathiazole or sulfamethazine were blown into both nostrils at each therapeutic procedure. This preparation was introduced twice a day for one-two-three days. During the outbreak of influenza in 1952 it was used in Leningrad by Tushinskiy and co-authors (1953), Kofman (1953), Yegorov and Novozhilov (1953), Morozkin and co-authors (1954) and others. They all expressed themselves in favor of this method of treatment. We have also noted the efficacy of the preparation (Table 112).

Under polyclinic conditions (Moscow, 1014 patients), the serum gave better results than sulfonamides or antipyretics. The Smorodintsev method of treatment, which is readily accomplished under house conditions, is promising and should be tried out in a large-scale epidemiological experiment. During the outbreak of A-prime influenza in 1953/1954 in Moscow the dry influenza Smorodintsev antiserum was used. It proved to be much more effective than the sulfonamides and antipyretics.

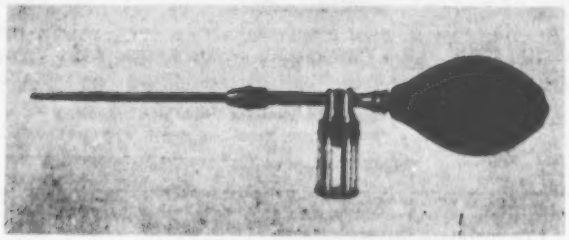

Fig. 256. Portable Powder-Blower (Original).

Table 112

Duration of Fever and of the Disease Depending on the Methods of Treatment During the Influenza Outbreak in 1952

Preparation and conditions of treatment	Total Number of patients	No. of patients with elevated temperature after beginning of treatment							No. of patients with pronounced disease picture						
		1–2nd day		3rd day		4–7th day		Average	3–4th day		5th day		6–7th day		Average
		total	%[1]	total	%	total	%		total	%	total	%	total	%	
Atabrine (hospital)	55	44	80	10	18	1	2	1,9	33	60	17	31	5	9	4,3
Control	31	16	52	3	10	12	38	2,9	14	45	3	10	14	45	5,3
Ecmoline (hospital)	91	60	66	19	21	12	13	2,3	48	53	18	20	25	27	4,7
Control	47	23	49	10	21	14	30	2,8	20	43	9	19	18	38	5,0
Atabrine (polyclinic)	40	30	75	9	22	1	3	2,1	18	45	21	52	1	3	4,3
Ecmoline (polyclinic)	36	16	45	12	33	8	22	2,8	13	36	10	28	13	36	4,9
Atabrine (medical unit)	31	10	32	2	7	19	61	3,7	2	6	9	29	20	65	6,2
Control	21	5	24	6	28	10	48	4,4	3	14	3	15	15	71	6,8
Dry Smorodintsev serum	46	37	81	8	17	1	2	1,9	29	63	8	17	9	20	4,5

[1] The data are presented arbitrarily in percentages for convenience of comparison.

According to the data of Yegorov and Novozhilov (1954), serum therapy was shown to be the most effective method of treatment of influenza (Table 113) in comparison with ecmoline therapy and symptomatic measures.

Table 113

Clinical Indices in Various Methods of Treatment (in Percentages of the Total Number of Patients in the Given Group)

Method of treatment	Temperature drop		Disappearance of intoxication		Disappearance of catarrhal signs		Clinical recovery		% of complications	Bed-days	Work loss (in days)
	No. of days	% of patients	No of days	% of patients	No of days	% of patients	No of days	% of patients			
Influenza antiserum	1	8	1	79	1	12[1]	1	0			
	2	84	2	17	2	68	2	13	None	3,7	5,3
	3	7	3	4	3	26	3	76			
	Over 3	1	Over 3	0	Over 3	4	Over 3	11			
Ecmoline intra-muscularly	1	0	1	57[1]	1	0	1	0	1,2	6,9	7,8
	2	71	2	35	2	12	2	0			
	3	25	3	29	3	29	3	11			
	Over 3	4	Over 3	2	Over 3	59	Over 3	89			
Ecmoline intranasally	1	0	1	43	1	0[1]	1	0		7,8	8,1
	2	65	2	46	2	15	2	0	1,9		
	3	23	3	6	3	22	3	13			
	Over 3	12	Over 3	5	Over 3	53	Over 3	87			
Symptomatic treatment	1	0	1	44	1	0	1	0		7,6	8,2
	2	34	2	49	2	34	2	0	1,4		
	3	49	3	5	3	38	3	20			
	Over 3	17	Over 3	2	Over 3	28	Over 3	71			

[1] The Table is taken from Yegorov's and Novozhilov's articles. Errors have been made in the Table.

Guzhienko and Morozenko (1953) obtained a favorable effect in children by treatment of influenza with influenza antiserum. After daily insufflations of the preparation two or three times, where treatment was begun early, the

temperature dropped to normal in the majority of children (Fig. 257), the signs of the toxic state soon disappeared, the catarrhal signs in the upper respiratory tract decreased and the number of complications dropped. Kofman draws approximately the same conclusions (1953).

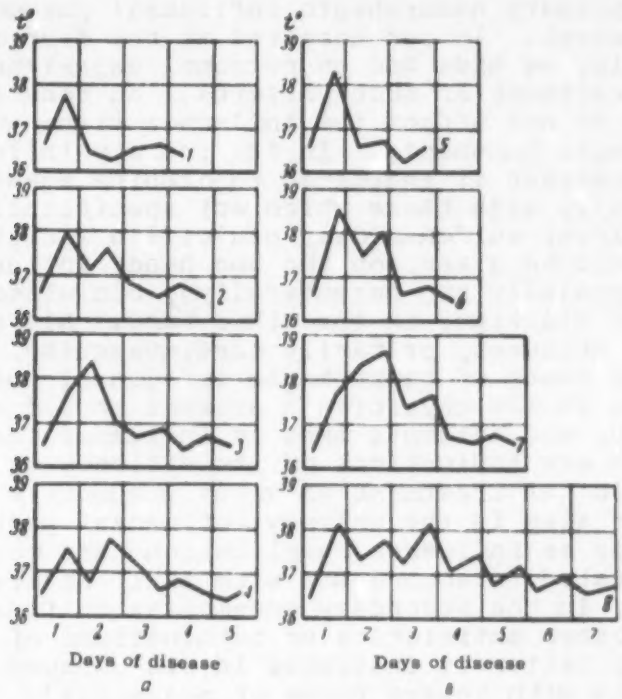

Fig. 257. Temperature Curves in Children Sick With Influenza and Treated With Dry Influenza Antiserum (a) and Symptomatic Measures (b) (Guzhiyenko and Morozenko, 1953).

Therefore, at the present time influenza antiserum, certain antibiotics and chemotherapeutic preparations which act on the influenza virus (atabrine, ecmoline, cutisone) or on the bacterial microflora (penicillin, sulfonamides) deserve the greatest attention in the specific therapy of influenza. These agents have been recommended in a collection on the treatment of infectious diseases (Gal'perin, 1953).

According to our data, the most effective measures for the treatment of influenza were Smorodintsev's specific polyvalent serum with penicillin and sulfonamides and

atabrine (particularly when used on the first day of the disease). After them come cutisone and ecmoline.

85. Treatment of Hemorrhagic Pneumonias and Influenza Complications

The primary hemorrhagic influenzal pneumonias are of special interest. In our hospital we had four cases, and, unfortunately, we have had no personal experience in the effective treatment of such patients. Sulfonamides and antibiotics do not affect the influenza virus which causes the hemorrhagic pneumonia. In the primary influenzal pneumonias we consider it indicated to combine agents which act antibacterially with those which act specifically on the influenza virus: sulfonamides, penicillin and strong antibiotics should be given, on the one hand; influenza antiserum (intranasally and parenterally), convalescent serum, ecmoline and atabrine, on the other hand. All available symptomatic measures, primarily cardiovascular, should also be used. In cases of hemorrhagic influenzal pneumonias all the measures in the physician's arsenal should be used comprehensively, and attempts made to influence the process.

There are indications of the efficacy of aureomycin (biomycin) in the treatment of virus pneumonias. It should be tried out also in the primary influenzal pneumonias.

As far as influenza complications are concerned, they are treated in accordance with their nature. Thus, for example, in the secondary pneumonias sulfonamides, penicillin and other antibiotics or combinations of them should be used (the latter is indicated in old persons and weakened patients with severe forms of pneumonia). Other complications (sinusitis, otitis, pyelitis, etc.) are treated appropriately depending on the form, nature of the disease and the condition of the patient.

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TENTH CHAPTER

PROPHYLAXIS OF INFLUENZA

86. Anti-epidemic Measures. Isolation of the Patients and Disinfection.

Epidemiological observations constitute evidence to the effect that in influenza the air-droplet route of transmission of the infection is the principal one. The closer and more intimate the contact with the source of the infection the higher the morbidity rate among those around.

This is confirmed also by experimental investigations on animals susceptible to influenza--ferrets. We should like to present an example taken from the work of Andrewes and Glover (1941), who found that the transmission of influenza A virus from infected to healthy ferrets occurs the easier the closer the cages containing the sick and healthy animals are to each other. The greatest distance at which transmission of the virus occurred was equal to 155 cm. It is important to note that the increase in the movement of air (ventilation) as well as the use of curtains, 120 x 80 cm. in size, prevented the spread of the infection among the ferrets.

The prophylaxis of influenza, like that of other infectious diseases, is based on three principles: the taking of measures directed at disinfection of the source of the infection and elimination of the virus in the environment; the taking of measures directed at increasing the resistance and creation of immunity to influenza in the population.

The first condition is fulfilled by means of early isolation of the sick from the healthy persons. It was noted long ago (Samoylovich, 1828) that even during most intense epidemics it was possible to avoid or postpone the importation of influenza into groups of people by means of separating them from the outside world.

Episodes of this kind, although isolated, were noted even during the pandemic of 1918. Thus, Andrewes (1939) reported that according to the material available to him, a severe outbreak of the disease occurred in the

southern part of the Pacific Ocean on the islands of Upolu and Savaii, during which 7,500 persons died from influenza of a population of 30,000 persons. On the neighboring island of Pago-Pago at this time an absolute quarantine was imposed on all ships sent into this port. As a result, the 1918 pandemic did not spread to Pago-Pago, and the inhabitants were protected against the importation of the infection.

Under modern conditions the success of quarantine measures can hardly be counted on, particularly since they have not justified themselves even previously, because of the difficulty in carrying them out. The example presented above is no more than an exception to this rule, which was favored by the location and the small territory of the island. Now measures are taken during epidemics for disinfecting the sources of infection by means of early detection of the patients and isolation of them chiefly at home. Patients with complicatied influenza are hospitalized. In housing and institutions in which many people live and in children's institutions special rooms--isolation rooms--are organized.

During the outbreak of influenza in the spring of 1949, regular observations were made on the efficacy of isolating patients as sources of infection to those around by Martsinovskiy and Samvelova (1950) under our direction. As a result of these observations it was shown that in a number of Moscow common dwelling places [the Russian term here includes places in which many people live, for example, apartment houses, dormitories, boarding houses, etc.] in which the detection and isolation of the patients were carried out correctly and on time the morbidity rate, on the average, amounted to 5.2 percent. In the common dwelling places, where these measures were not taken at all or where they were taken incorrectly, the morbidity rate reached 27.6 percent.

Ritova (1951, 1953) presents similar data; she believes that the measures for the protection of children against infection are a particularly important factor in the prophylaxis of influenza. She presents a number of examples which indicate the fact that timely and complete isolation of the patients from healthy persons reduced the incidence of influenza in the children's institutions to 50-70 percent. In the work of Gaydamaka and co-authors (1952) the efficacy of the simplest isolation measures for influenza patients has been shown in the common dwelling places for industrial workers, as well as the efficacy of putting children in nurseries night and day during the influenza epidemic.

There is a cruder system of isolating sources of

infection which has an influence on the influenza morbidity rate in foci of influenza. According to Martsinovskiy and Samvelova, in families and in apartment houses where the patients were separated from healthy persons in a separate room and also by means of placing screens or hanging drapings made of sheets around them, the persons around became sick in 34 percent of the cases as against 65.3 percent in families and apartment houses where such precautionary measures were not taken.

The early isolation of the patient is essential, because his contagiousness is greatest during the first three days of the disease. Those taking care of the patients may become infected themselves and may serve as the cause of infection of others. This is why wearing of protective dressings or masks made of gauze is recommended. The dressing is applied to the face so that it covers the mouth and the nose. The gauze in the dressing should be multilayered and should first be washed out several times: in gauze which has been repeatedly washed the permeability is reduced because of the tousled state of the fabric.

According to the data of Vashkov (1954), masks which consist of two layers keep back from 55-79 percent of exhaled microorganisms; masks composed of three layers, up to 80 percent; of four layers, from 90-94 percent; and masks made of six layers keep back up to 97 percent.

Of the organizational measures which assure the timely isolation of the patient isolation should be provided in children's and adolescents' institutions of the hospital type (children's homes, infants' homes, children's nurseries and kindergartens, boarding schools) as well as in common dwelling places for students, workers, etc. by means of isolation rooms where the patients should be kept.

Another measure of an organizational nature, which assures a partial isolation of sick from healthy patients, is extra-hospital aid at home for patients with influenza and the proper organization of the work of polyclinics, particularly during periods that are epidemiologically unfavorable for influenza.

The measures of controlling influenza which are directed at isolation of the source of the infection can be supplemented by disinfection of the air in closed quarters.

Transmission of influenza by droplet nuclei is not of any essential epidemiologic importance, but it should, nevertheless, be taken into consideration for the proper formulation of antiepidemic measures (Edward, 1941; Vashkov, 1953). The survival of the virus in the environment is not very great; however, one should keep in mind the results of experimental investigations of infectious mate-

rial in the form of lung tissue extracted from sick mice. When woolen blankets are dipped into a five-percent suspension of mouse lung tissue with subsequent drying at room temperature, according to Edward's data (1941), the virus may be preserved up to two weeks and it can enter the air with the dust when the blankets are shaken out. According to the observations of Vashkov (1953), a dried suspension of lung tissue applied to glass maintains its infectivity up to twelve days; when applied to wall paper, from three to nine days; to a wooden surface, from one to three days.

In evaluating experimental data of this kind it should be kept in mind that a suspension of mouse lung tissue infected with the influenza virus contains billions of minimum lethal doses for these animals. Mice have also served as the indicator of survival of the virus. In addition, the viability of the virus in the lungs during drying is maintained by a dense protective tissue layer. Therefore, the artificial nature of such experiments is obvious; it does not correspond to natural conditions. Under natural environmental conditions when all the conditions are favorable the survival of the virus is limited to hours rather than days. At the same time, these data confirm the necessity of heat treatment of glass vessels and linen which are used by the patients and which have been contaminated by their excretions. For this purpose, simple boiling is adequate, and this rapidly kills the virus; for objects which cannot be boiled washing with soap solution may be recommended. During epidemic outbreaks of influenza glass vessels should be treated with boiling water in all public eating places.

For the purpose of disinfecting the air in closed quarters a number of chemical agents was tried out, of which the most effective under experimental conditions were aerosols obtained from solutions of lactic, pyruvic, levulinic, glyceric, dichloracetic, trichloracetic and monochloracetic acids. In experiments on mice, using a concentration of these substances of 10 mg/cubic meter, they possessed a sterilizing effect on a sprayed virus suspension (mouse lung suspension) and reduced the morbidity rate of the animals to the extent of 92 percent (Vashkov, 1950, 1953). An even stronger effect on the sprayed suspension was exerted by glycol aerosols. According to the data of Henle and Zellat (1941) and Vashkov (1953), the concentration which destroys the virus is produced by the evaporation of one gram of triethylene glycol per 100-200 cubic meters of air. Aqueous halogen solutions possess the strongest effect on the influenza virus when suspended in the form of a mist or when dried and sprayed (Lush and Burnet, 1941).

Despite the fact that the possibility of effective use of chemical disinfection of the air has been proved, the application of this method still is experimental. As a matter of fact, disinfection of the air for the prophylaxis of influenza should be carried out in the presence of people; otherwise, it is not of any practical importance. Therefore, the first requirement made on the preparations used is that they must be completely harmless for a long time. In addition, they should not possess any odor, should not spoil articles and should not be inflammable. The entrance of them into the air should be uniform and accurately dosaged, for which it is necessary to have an appropriately designed apparatus suitable for mass use.

The circumstances listed led to the fact that the majority of attempts to incorporate air disinfection into practice have been made with a physical method, more accurately ultra-violet irradiation, rather than with a chemical one.

There are a number of reports on the effect of ultra-violet rays, beginning with the work of Wells and Brown, 1936. These investigators found that virus suspended in the air of a closed chamber in the form of a suspension of mouse lungs became noninfectious for ferrets after a ten-minute irradiation with a quartz lamp.

Afterwards, it was established that the strongest bactericidal effect under conditions of air disinfection was shown by ultra-violet lamps with a wave-length of 2,537 Å units with an intensity of the effect of 20 microwatts per square centimeter. Such lamps, in which the quartz has been replaced with uviol glass, are being produced by Soviet industry and have been applied to the sterilization of air in cubicles and in operating rooms. A number of experiments has been published on the use of such lamps with the aim of prophylaxis of infections of the upper respiratory tract generally and of influenza in particular. Thus, Castoldi (1953) observed a halving of the incidence of air-droplet infections among 600 students in irradiated classes during the three winter months in one of the Milan schools. For the purpose of obtaining this result the lamps were operating five hours a day, that is, during the entire time that the students were in the classes. We must direct attention to this fact because the use of ultra-violet irradiation, like any other method of air disinfection, can be effective only in the presence of people filling the room.

Unfortunately, in this and in other similar work there are no proofs of the harmlessness of continuous irradiation. Nor are there any control observations of the frequency of disease in rooms in which a careful hygienic

routine is carried out: systematic moist cleaning and a well operating ventilation system.

We believe that the best and most available measures at the given stage of practical utilization of the possibilities of air disinfection for the prophylaxis of influenza are the simplest hygienic measures, which are particularly important for industrial enterprises, schools and children's institutions. They include a properly constructed ventilation system and frequent airing of the rooms, a good system of moist cleaning directed at the elimination of dust and the abundance of sunlight in the room. To what has been said, measures should be added which are directed at the dispersion of people, at the elimination of closeness and crowding.

Chlorine preparation solutions (chloride of lime, chloramine) have been used for a long time during epidemic outbreaks of influenza for moist cleaning of the rooms. At the recommendation of Okunevskiy (1936), 0.2 percent chloride of lime solution is most often used according to the calculation of 0.3 liter per square meter of area of the floor. Thereby, gaseous chlorine is released into the air in a concentration which destroys the influenza virus (Smorodintseva, 1953).

Of the other organizational measures for the prophylaxis of influenza we should point out the taking of hygienic measures in factories, schools and under ordinary living conditions. Despite the evidence of great benefit of these measures in the prophylaxis of influenza, there are no investigations in the recent literature which contain an objective evaluation of them, despite the fact that there is no dearth of statements concerning their benefit (Rossiyskiy, 1949).

Since these measures are of a general sanitary nature and are not particularly directed against influenza, we consider it necessary to discuss them in greater detail. Therefore, we are directing attention to the particular importance of such hygienic measures as the observance of sanitary standards for the volume of factory, school and living quarters, the proper arrangement of ventilation, which provides an adequate exchange of air and elimination of harmful admixtures (dust, gases) as well as the optimum temperature routine and humidity in the prophylaxis of influenza. This group of hygienic measures is most important in the prophylaxis of influenza.

87. Nonspecific Methods of Prophylaxis.

Many different methods and measures have been pro-

posed for the prophylaxis of influenza, which, however, have not obtained recognition. Among them, particularly, is the so-called "influenza antivirus" (Fal'kovich, 1939), which represents a filtrate of a bouillon culture of the Afanas'yev-Pfeiffer Bacillus. Gamaleya (1942) suggested smearing the nasal openings with soap-suds in the belief that the associated increased production of secretion of the mucous membranes creates a local resistence to influenza.

The preparations and methods which have been studied most extensively are the following: rinsing of the throat with various antiseptic solutions (potassium permanganate, boric acid), the prophylactic ingestion of urotropine and a preparation of it (Mashkov, 1938), prophylactic administration of small doses of iodine after Schindler (Rossiyskiy, 1940), the taking of fish oil and other vitamin preparations (Rossiyskiy, 1949).

The effect of ultra-violet irradiation and of small doses of chlorine on the influenza morbidity rate has been studied in particular detail (Lipkovich, 1936; Rossiyskiy, 1940, 1942, 1949). In Rossiyskiy's monograph data are presented (Fig. 258) which indicates a reduction of the influenza morbidity rate among persons exposed to irradiation with a quartz lamp. However, authors everywhere speak of "influenza" without differentiating it from acute catarrhs by means of virological and serological investigations. It must be supposed that in these cases quartz irradiation as a method for increasing the general resistance of the body exerted an influence chiefly on the incidence of acute catarrhs and other diseases of this group. The justifiability of this conclusion has been confirmed by Lipkovich's data; he points out directly that irradiation of patients who frequently have influenza reduced the morbidity rate among them. Apparently, the administration of vitamins in the spring-time has the same significance. There are no definite proofs of the prophylactic effect of quartz irradiation on the influenza morbidity rate in any of the works mentioned (we are not talking about disinfection of the air here, which was mentioned above, but rather of increasing the resistance of people to influenza through the influence of ultra-violet irradiation).

Even larger is the literature on the problem of chlorine prophylaxis of influenza by means of putting people into a chamber the air of which is saturated with low concentrations of gaseous chlorine, a review of which has been presented in Rossiyskiy's monograph (1945). The basis for the use of this method of controlling influenza is constituted by observations indicating the low influenza morbidity rate among workers in the chemical indus-

try. The contradictory results obtained through the use of this method were apparently explained by the inadequately strict epidemiological observations. Experimental verification (Smorodintsev, Shishkina, 1937; Smorodintseva, 1953) has shown that chlorine does not increase the resistance of animals to influenzal infection although it exerts a bactericidal effect on the influenza virus sprayed into the air.

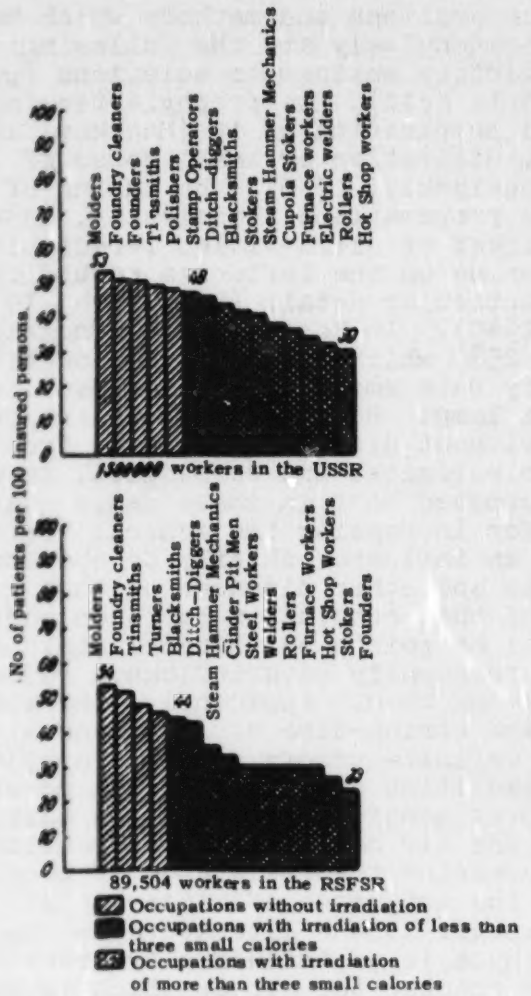

Fig. 258. Influenza Morbidity Rate Among Workers in the Machine-Construction and Metallurgical Industries in the USSR, Exposed to Ultra-Violet Irradiation (Rossiyskiy, 1949).

In summary, all the methods of nonspecific prophylaxis of influenza mentioned have been rejected as theoretically unsound and practically unjustified.

Attempts at using various chemotherapeutic preparations and antibiotics for prophylaxis have not given any positive results either. Thus, in the Naval Laboratory Unit (1943, No 1) in the United States tests of 15 sulfonamides were made (including sulfadiazine, sulfathiazole and sulfapyridine), of three acridines (including rivanol and trypaflavine), and of five antibiotics (including penicillin and gramicidin), which proved to be ineffective in influenza. The carefully performed experiments of Dzagurov (1953) showed the absence of any prophylactic effect of synthomycin, levomycetin, penicillin, gramicidin or aureomycin in influenza.

The existing data concerning the efficacy of the prophylactic effect of atabrine (Gal'per, 1953) require checking in a more serious manner.

Among the nonspecific (non-immunological) methods of influenza prophylaxis is the use of ecmoline. This preparation, an antibiotic of animal origin obtained from fish milt by Yermol'yeva and Valedinskaya (1952), was tested out for the prophylaxis of influenza in people after an experimental study was made by Shubladze and Gaydamovich (1952). According to the data of Lukashevich and Zak (1950, 1952), Ritova (1951, 1953), Nikolayev, Lev and Slepushkin (1952, 1953), the intranasal administration of ecmoline reduces the influenza morbidity rate by 1½ to three times, and in the patients it shortens the course of the disease somewhat. For the purpose of obtaining the maximum effect, ecmoline is used repeatedly, every three-four days, throughout the entire time set apart for prophylaxis. Ecmoline is instilled into the nose in the form of drops in a dose of 0.4-0.8 cubic centimeter. Gal'per (1953) obtained less demonstrative results.

The greatest number of works has been devoted to the immunological prophylaxis of influenza. Protective immunization of the population is well grounded theoretically and, as has been shown in recent years, may be introduced into the general practice of anti-epidemic measures. We believe it expedient to present the results of the investigations made in this direction in greater detail, grouping them in three main divisions: 1) killed (inactivated) influenzal vaccines; 2) live influenzal vaccines; 3) the serum prophylaxis of influenza.

88. Inactivated (Killed) Influenza Vaccines.

Attempts at creating an immunity against influenza

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by means of artificial inoculation of the inactivated virus in man were undertaken soon after its discovery. Four successive phases in the development of work in this direction should be noted: 1) the preparation of the vaccine from the lung tissue of infected mice; 2) the preparation of the vaccine from the allantoic fluid of chick embryos; 3) the preparation of concentrated vaccines from allantoic fluid; 4) the preparation of vaccines with so-called "adjuvants" or stimulators.

The immunogenic effect of the inactivated vaccines has been studied most often after their subcutaneous inoculation; in addition, such vaccines have been given to people intracutaneously and intranasally.

Andrewes and Smith (1937), Francis and Magill (1937) and Smorodintsev (1938) showed that the subcutaneous injection of formalinized filtrates of mouse lung tissues infected with the influenza virus produces a considerable rise in antibody titer in vaccinated persons. The antibodies also increased in the nasal secretion (Smorodintsev, Chalkins and Gulamov, 1945; Francis and others, 1943).

The highest antibody level in the blood of volunteers was found by these authors two weeks after the vaccination. After that, the antibody titer began to drop gradually, being maintained at a high level for two months. In the next four or five months the antibody titer in the blood gradually decreased to the original titer. The authors mentioned, as had Chalkina (1938), noted that the increase in the antibodies in the blood of those inoculated was higher after the vaccination the lower the titer of them in the blood before vaccination.

Stuart-Harris, Andrewes and Smith (1938) made similar observations during the epidemic of 1936-1937, and, in addition noted that the immunization did not reduce the morbidity rate among those vaccinated.

The authors ascribed this failure to the antigenic incompleteness of their vaccine and suggested that some kind of "basic" strain exists in nature the incorporation of which into a vaccine would assure protection for those immunized against all other virus strains. Therefore, they recommend finding "the basic" strain and including it in a vaccine.

After these first attempts at using formalinized vaccine for immunization of people against influenza a number of reports of American authors was published, concerning the so-called "comprehensive vaccine", which was supposed to possess a high degree of antigenic activity. Horsfall, Lennette, Rickard and Hirst, Sigel and others (1942), Bauer and Nickels (1940) found that if the virus

of distemper is added to the influenza antigen this comprehensive vaccine is much more effective.

As a result of experiments on ferrets and the determination of the rise in antibody titer in immunized people the authors came to the very promising conclusion that the comprehensive vaccine was "at least as effective in producing immunity as having had the natural disease".

The comprehensive vaccine was given once subcutaneously to 7,907 persons and there were 9,688 controls. Four months after the vaccination an epidemic of influenza A broke out, during which the morbidity rate among those inoculated was equal to 7.4 percent and among the controls, to 9.8 percent. In other observations (Dalldorf and others, 1941) the vaccination one and a half to two months before the influenza outbreak did not reduce the morbidity rate at all among those who had been inoculated (Brown and others, 1941; Martin and Eaton, 1941).

Despite the fact that serological investigations showed the correlation between the influenza virus strain included in the vaccine and the strain participating in the epidemic outbreak, the authors' expectations were not justified on epidemiological checking, and afterwards the comprehensive vaccine was not used in practice. After these experiments on the study of formalinized subcutaneous vaccines in which an immunological effect had been obtained but in which no reduction in the morbidity rate was achieved in those inoculated no extensive epidemiological testing of this kind of vaccine was carried out. Only in 1943-1945 did Ansheles (1945, 1950), and Orlova (1946 and 1948) immunize people with a combined vaccine, which included, in addition to the formalinized influenza antigen prepared from mouse lung tissue, triple vaccine, dysentery vaccine and tetanus toxoid. Ansheles, at the beginning of the 1943 epidemic in Leningrad, used a mixed vaccine of this kind which included the PR8 and Leningrad strains.

The inoculations were given three times subcutaneously at five-day intervals in a dose of one-two cubic centimeters of the mixed vaccine. In all, 1,666 persons were included in the inoculations; the control group was made up of 1,446 persons. Side-reactions from the inoculations were moderate. A decrease in the morbidity rate by two to two and a half times in those inoculated was noted beginning with the tenth day after the start of vaccination. Orlova (1948) used the mixed vaccine for the purpose of giving inoculations to students in trade schools (1,000 persons). Those included in a control group were inoculated with a similar vaccine which did not include the influenza antigen. General nonspecific side-reactions from the mixed vaccine were noted in 4.8

percent of those inoculated. The efficacy of the vaccination was determined during the influenza outbreak in January-February of 1946, ten months after the inoculation. Six hundred and forty-one persons were included in the experimental group; 617, in the control. The mixed vaccine led to a reduction in the morbidity rate among those inoculated by two and a half times and reduced the average duration of the disease by one and a half days in the vaccinated persons.

In 1948, Chalkina (1949) suggested eliminating the inert tissue components from the mouse lung vaccine by means of supercentrifugation or filtration through colloidal membranes. However, after the valuable method of obtaining large quantities of virus through culturing it in chick embryos appeared, vaccines from lung tissues lost their importance and are now no longer used.

For the purpose of immunizing people, concentrated vaccines prepared from the allantoic fluid of infected chick embryos have been used most extensively. For the purpose of concentrating the virus from the allantoic fluid several methods have been suggested of which the most frequently used are adsorption onto erythrocytes with subsequent elution of the virus in a reduced volume of buffered saline solution (Francis and Salk, 1942) and precipitation of the virus by means of differential supercentrifugation. The latter method was developed first by Elford and Andrewes (1936), and then by Stanley (1944, 1945, 1946) and Taylor and his co-workers (1945), Knight (1946), Loosli and co-authors (1948).

Stanley believes that this method is most productive when compared with the others, which leave up to 80 percent of the non-virus protein in the preparations, and makes it possible to concentrate the virus maximally after it has been purified from its nonspecific tissue components. In evaluating supercentrifugation as the best method of obtaining the purified antigen, Stanley recommended it for mass production of influenzal vaccine. It should be noted that the conclusions of Stanley concerning the high degree (up to 90 percent) of virus purification in the antigen obtained by means of supercentrifugation have not been confirmed by other investigations.

Thus, Knight (1946) found that this concentrate contains no less than 30 percent of the normal tissue proteins characteristic of a chick embryo allantoic fluid. It is important to emphasize also that the antigen obtained by means of supercentrifugation produces a considerable number of nonspecific general and local reactions when injected into people subcutaneously. They are particularly pronounced when the protein concentration in the vaccine is

increased to more than 0.3 mg/cc. Of the other methods of preparing the influenzal vaccines, methods of virus adsorption from the allantoic fluid onto calcium phosphate and sluminum phosphate have been quite extensively utilized (Henneberg and Wengel, 1953).

Inactivation of the virus in vaccines and protection against bacterial contamination was usually accomplished with 0.05 percent formalin solution, less often with ultraviolet irradiation. Usually weak solutions (0.002 percent) of phenylmercuric preparations such as merthiolate or phenylmercuric nitrate are added to the vaccines.

It should be noted that the advantage of the vaccine prepared according to the Stanley method of high-speed centrifugation has not been confirmed in immunological investigations either. Thus, Loosli, Schoenberger and Barnett (1948) showed that the vaccines composed of three strains, PR8 (25 percent), WS (25 percent) and Lee (50 percent), prepared by various methods give similar results and in any case do not speak for the advantage of the vaccine obtained by means of supercentrifugation (Table 114).

Table 114

Average Antibody Titer Before and After Subcutaneous Injection of One Cubic Centimeter of Vaccines Prepared by Different Methods

Method of preparing the vaccine	No of paired sera investigated	Virus strains	Antibody titer	
			before vaccination	after vaccination
Adsorbed with calcium phosphate and inactivated with ultraviolet irradiation	94	PR8	374	1 944
	95	WS	224	1 075
	111	Lee	156	512
Concentrated on erythrocytes and inactivated with formalin	97	PR8	294	1 075
	94	WS	137	914
	115	Lee	114	433
Concentrated by centrifugation and inactivated with formalin	90	PR8	255	806
	95	WS	182	530
	93	Lee	104	465

Henle and co-authors (1943) attempted to clarify the efficacy of the formol vaccine from allantoic fluid in experiments on people. For this purpose a vaccine was pre-

pared with which children were immunized; after that, they were infected with the A (99) virus which had been isolated from a child who died and which had been passaged through mide and chick embryos. When it was given by means of inhalation to unvaccinated children the virus produced a picture of influenza and could be isolated from the nasopharynx. In the majority of those inoculated no disease developed. Therefore, the vaccine was effective.

The epidemiological study of subcutaneous concentrated vaccines was begun in the United States in 1943. Inoculations were given with a vaccine which had been concentrated by adsorption onto erythrocytes with subsequent elution and which included three antigens: the PR8 and Weiss strains (isolated in May 1943) of type A (50 percent) and the Lee strain of type B (50 percent). The vaccine was given subcutaneously once in a dose of one cubic centimeter. As a control, an equal dose of isotonic sodium chloride solution containing formalin and phenylmercuric nitrate was given by the same route.

There were 12,474 service men under the observation of a special Commission on Influenza of the Army Medical Service. According to the report of this Commission (1944) and of Francis (1947), vaccination was given at various times but for the most part was concluded in November 1943. An epidemic of influenza A began in the second week of November and continued for a month.

The nonspecific reactions to the injections of vaccine were moderate. Serological investigations made in part of the vaccinated persons showed a rise in antibody titer in the blood of persons who had low indices before vaccination. The morbidity rate was recorded on the basis of clinical diagnosis. Among 6,263 inoculated persons the total morbidity rate amounted to 2.22 percent; among 6,211 controls, 7.22 percent.

Therefore, the vaccination led to a reduction in the morbidity rate by an average of 3.2 times. At various installations the reduction amounted to from one and a half to six times. This depended on the timeliness of giving the inoculations as well as on the conditions under which the experimental and control groups were (Hirst, Rickard and Friedewald, 1944; Hale and McKee, 1945; Magill and others, 1945).

During an epidemic of influenza type B in the winter of 1945 a number of American authors also reported success in subcutaneous inoculations with concentrated allantoic vaccine. According to the data of Francis, Salk and Brace (1946) 600 persons were inoculated three weeks before the outbreak. Among those vaccinated the morbidity rate amounted to 1.15 percent; among 1,110 controls, 9.11 percent.

Therefore, an eight-fold reduction in the morbidity was observed. Similar data were obtained by Hirst (1947), Norwood and Sachs (1947) and Francis (1947).

After a number of optimistic reports by American authors on the successful use of inactivated subcutaneous vaccines failure overtook them in the study of the efficacy of this method during the epidemic of 1947.

During the autumn-winter period of 1946/1947 a number of military, school and industrial groups were vaccinated, and in March 1947 a widespread influenza epidemic broke out which included almost all the states.

Francis, Salk and Quilligan (1947) vaccinated 10,328 persons using 7,615 controls and did not find any prophylactic effect in connection with the vaccination. Baetjer (1948) made observations on inoculated workers of 13 factories located in different areas of the United States and noted a certain reduction in the morbidity rate at six of the factories without any positive results in the others. Sigel and others (1948) inoculated 464 persons and van Raavensway (1948), 237 students and also obtained a negative vaccination result. In the former case, the morbidity rate among those inoculated amounted to 54 percent; among those not inoculated, 49 percent; in the latter case, 20 percent of those inoculated became sick; in the control group, 27 percent. Loosli, Schoenberger and Barnett (1948) vaccinated 790 students, and the morbidity rate among them amounted to 9.5 percent, whereas the same figure was found among 1,230 unvaccinated persons.

Similar results were obtained also in other observations particularly among military groups.

The cause of the failure of the inoculations lay in the marked differences in the antigenic structure of influenza A virus strains included in the vaccine from that of the newly isolated A-prime virus which was the cause of the influenza epidemic. In explaining the absence of any effect of the vaccination by this, the authors indicated the need for making additional investigations for the purpose of determining the possibility of further use of the vaccine and the elimination of its inherent defects. Specifically, Rasmussen, Stokes and Smadel suggested that new specially islated strains be included in the vaccine for the purpose of improving it.

English investigators also ran up against the fact of the poor prophylactic effect of subcutaneous immunization with concentrated vaccines (Stuart-Harris, Andrewes and others, 1947, 1948).

Unsuccessful experiments in 1946/47 reduced the interest of foreign investigators in the problem of vaccination against influenza. Large-scale epidemiological experi-

ments were carried out in the United States and in England only in 1950 and 1952. During the period from 1947 to 1949 a study was made of the possibilities of increasing the efficacy and decreasing the cost of the vaccines by means of substituting the intracutaneous method for the subcutaneous method or by means of using so-called "stimulators".

Bruyn, Meiklejohn and Brainerd (1949) showed that both the subcutaneous (one or two cubic centimeters) and the intracutaneous (0.1-0.2 cubic centimeter) injection of the vaccine produced the same rise in antibody titer in the blood of adults.

Wolmer (1950) injected 0.1 cubic centimeter of the formalinized vaccine intracutaneously which had been concentrated by centrifugation into 123 children from 2-16 years of age; in 21 of them an enlargement and edema of the regional lymph glands were noted; in three, a general febrile reaction.

Salk (1947, 1948) studied a vaccine prepared from a virus which had been adsorbed by calcium phosphate. Half of the quantity of virus adsorbed onto the calcium phosphate produced the same immunity as double the quantity of unadsorbed virus. On the basis of the maintenance of a high antibody level in the blood the author believes that post-vaccinal immunity remains for seven months.

Schwarz-Tiene (1950) observed the immunological reactions in 45 children in response to a subcutaneous or intracutaneous injection of the purified aluminum-phosphate-adsorbed virus. He injected 0.5 cubic centimeter of the virus subcutaneously or 0.1 cubic centimeter intracutaneously. A local inflammatory reaction was noted only in children who had been inoculated intracutaneously; in one of them there was a general reaction. No conclusion was drawn as to the advantage of either method of vaccination over the other. Cohen and Schneck (1948) vaccinated 100 children subcutaneously with calcium-phosphate-adsorbed vaccine; the children were from one month to 16 years of age.

A considerable rise in the antibody titer was noted in children over two years of age with a reduction of the morbidity rate by 3.2 times. Stuart-Harris and co-authors (1951) studied aluminum-phosphate-adsorbed vaccine in small groups of inoculated persons as well as a vaccine which had been concentrated on erythrocytes and which was injected subcutaneously in a dose of one cubic centimeter and intracutaneously in a dose of 0.1 cubic centimeter. The authors believe that the former of them is more economical and produces fewer vaccinal reactions. The rise in antibody titer was greater (by two to two and a half times) after subcutaneous vaccination.

The influence of repeated injections of the vaccine

adsorbed onto aluminum-phosphate in producing influenzal antibodies in immunized people was determined by Schäfer, who showed that the second subcutaneous injection (after an interval of a week) increases the titer; the third is not effective. A comparison of the intensity of the rise in antibody titer after subcutaneous and intracutaneous injection of formalinized influenza vaccine was made by Bucco, Colucci and Jacono (1951). They found that adults inoculated subcutaneously with one cubic centimeter of the vaccine (62 persons) showed a much more intense rise in antibody titer after the vaccination than those who had been inoculated intracutaneously with 0.1 cubic centimeter of the vaccine (60 persons). An attempt to increase the dose of the vaccine given by subcutaneous injection to 0.5 cubic centimeter did not increase the immunological reactivity, but led to serious local and general nonspecific reactions.

In one of their works (1952) they note an advantage of the intracutaneous method over the subcutaneous, based on a comparison of the antibody titer figures in the blood.

The great laboriousness of this method, however, and the considerable number of local as well as general vaccine reactions which occurred in those inoculated evoked doubts as to the possibility of its extensive application. The differences in the conclusions of authors who studied this problem (Weller and others, 1948; Gelder, 1947) also attract attention.

Beginning with 1950, the results of observations devoted to the study of the efficacy of subcutaneous concentrated influenza vaccine were published again in the United States.

Eddy (1952) in November-December 1950 studied the efficacy of the commercial vaccine which included the FM1 (25 percent), PR8 (12.5 percent), Lee (12.5 percent) and Bon (50 percent) strains. The vaccination was accomplished by means of subcutaneous injection of 0.5 cubic centimeter of the vaccine. In recording the morbidity rate the diagnosis was made on the basis of the clinical picture, and the latter was usually established on the basis of interrogation of those who had had the disease following their recovery rather than by means of direct examination of the patients. The vaccination was carried out at an industrial enterprise. In all, 1,148 persons were vaccinated; the control group included 2,349 persons. Among those vaccinated 124 became sick (10.8 percent); among the controls, 353 (15.02 percent). The author explains the low degree of efficacy of the vaccine by the differences in the antigenic structure of the epidemic strain and the strains included in the vaccine and draws the conclusion that it would be ideal for the purpose of increasing the efficacy of the vac-

cine to include in it a strain which had been isolated at the beginning of this epidemic.

In January-February 1950, during an epidemic of A-prime influenza in California, Lennette and co-workers (1952, 1954) made observations on the immunization of several groups of persons totaling 3,687 with a monovalent vaccine; there were 1,784 persons in the control group. The morbidity rate figures were the following: in the group of those inoculated with the PR8 vaccine, 3.1 percent; in the group of those inoculated with FM1 vaccine, 1.2 percent; and in the group of those inoculated with the vaccine prepared from the Lee strain, 4 percent. In the control unvaccinated group, 4.2 percent became sick. As is seen from the figures presented, a distinct reduction in the morbidity rate was obtained only in those who had been inoculated with the antigen (FM1) which in its type coincided with the virus producing the epidemic.

In another experiment, where monovalent B vaccine was inoculated into 2,596 persons, A-prime vaccine into 2,600 persons and 2,628 persons remained as controls during an influenza A-prime epidemic, 1.9 percent became sick in the first group; 0.5 percent in the second group; 2.1 percent in the third group. Even in this case the presence of the A-prime component in the vaccine assured its efficacy during the outbreak of A-prime influenza.

During an influenza epidemic in Basel in January 1951 Bernstein, Reber and Sheris (1952) vaccinated 536 medical workers and concluded their inoculations before the maximum rise in morbidity rate. During the first ten days after the vaccination the morbidity rate among those inoculated amounted to 3 percent and to 3.2 percent among 405 unvaccinated persons. During the next five months of the observation this difference was expressed more distinctly: 4.5 percent became sick among those vaccinated and 8.4 percent among the unvaccinated.

In this work the data on the nonspecific reactivity of those inoculated are interesting. General reactions were noted most often in those inoculated by the intracutaneous route: in the group of those inoculated once, 37 percent; in the group of those inoculated twice, 33 percent. Reactions at the site of injection of the vaccine (85-94 percent) were particularly frequent. Subcutaneous inoculations of a similar vaccine adsorbed by aluminum-phosphate produced 23-percent general and 48-percent local reactions. The authors indicate that even five months later traces of the inoculation remained unabsorbed in the form of nodules and sterile pustules which were found in 70 percent of those who had been inoculated intracutaneously once and in 83 percent of those who had been inoculated intracu-

taneously twice.

The Italian investigators Giordano and Pino (1953) tested out the intracutaneous injection of polyvalent concentrated vaccine. They found that among the inoculated persons (41 persons) who had been given an intracutaneous immunization with 0.2 cubic centimeter of the vaccine, just as among 37 persons vaccinated subcutaneously with one cubic centimeter of the vaccine, there were no severe reactions, and they noted that the latter produced a higher rise in antibody titer.

A vaccine concentrated by aluminum-phosphate was tested in England in the winter of 1952/53. According to the report of the Medical Research Council (1953) this vaccine which contained the FM1 strain and the Liverpool strain was compared with the control polyvalent bacterial vaccine prepared in a similar way. The influenza vaccine was inoculated into 6,340 persons; there were 6,370 controls. The figure for the morbidity rate in the first group anounted to three percent; in the second group, 4.9 percent.

In Holland in November-December 1952, Verlinde, Makstenieks and Nass (1954) carried out anti-influenzal inoculations with three types of vaccine containing different strains of influenza viruses. The maximum rise in the morbidity rate was observed in February 1953. The results of the vaccination were recorded among 14,583 inoculated persons compared with 12,456 controls; a slight reduction in the morbidity rate was noted here only in the groups which had received the first and third vaccines which contained the A-prime antigens. The serological investigations showed that there were antibodies against the freshly isolated virus strains in the blood of persons immunized specifically with these vaccines.

Francis and co-workers (1953) vaccinated two groups of children with concentrated monovalent antigens A-prime and B during an outbreak of influenza B in the winter of 1952. A reduction in the morbidity rate by 2.7 times was noted only in the group of those inoculated with type B vaccine.

In generalizing on the material presented above and other published material on the study of the efficacy of subcutaneous immunization with inactivated vaccines it may be considered that they reduce the influenza morbidity rate by an average of two or three times. Such an effect was observed only when the inoculations were used on time and in the event strains were included in the vaccine which were similar in their antigenic structure to the virus causing the given epidemic outbreak. The intracutaneous method of immunization has not been extensively applied in connection with the frequency of nonspecific reactions in those

inoculated and the complexity of giving the inoculation. In epidemiological experiments of testing the prophylactic value of vaccines foreign investigators ran across two principal obstacles in solving the problem of inoculation prophylaxis of influenza. First, the change in the types of virus and the antigenically different qualities of the strains isolated, which served as the reason for a number of failures in giving the vaccination; and, secondly, the inadequate strength of the immunity which occurred after the vaccination.

It should be added also that after subcutaneous injection of concentrated antigens consisting of both the virus and egg white allergic reactions cannot be excluded. Ratner, Untracht and Hortzmark (1946, 1952) direct attention to this fact.

For the purpose of improving the quality of inactivated vaccine a number of investigators, like those mentioned above, suggested including new antigens prepared from freshly isolated influenza virus strains. However, such authors, like, for example, Bernstein (1952) suggested preparing the vaccine from strains isolated during a given epidemic. Such a suggestion cannot always be carried out because isolation of the virus, identification of it and adaptation to chick embryos, and then mass production of the vaccine and the immunization process require considerable time. In addition, it is known that vaccination carried out at the height of the epidemic does not contribute much to reducing the morbidity rate.

Certain authors attempted to improve the quality of the vaccines by means of adding different mixtures to them (Friedewald, 1944).

Salk and co-workers (1947, 1951, 1952, 1953) suggest improving the quality of immunization by means of introducing various admixtures into the vaccine which influence immunogenesis and stimulate antibody formation. In making the study of the vaccine adsorbed onto calcium-phosphate, which creates a depot of the antigen whereby the duration of the antigenic stimulation is increased, he was not satisfied with its effect and he changed over to observations on a vaccine with a different kind of admixture. Specifically, inactivated concentrated allantoic vaccine emulsified in "Arlacel AA" with an admixture of mineral oil "Biol G" was studied and gave good results in experiments on animals. This vaccine was studied in the immunological experiments of Pontecorvo and Picciotto (1953). The authors believe that the influenza A and B viruses possess a definite antigenic structure, and all the changes and differences in the various influenza virus strains are variations of this structure. Therefore, by increasing the immunogenicity of

the vaccine considerably with respect to these principal antigens it is possible, supposedly, to obtain an immunity against the most varied strains existing at present and possible in the future.

With respect to this it should be noted that the authors were too optimistic in their suggestion. Actually, a high degree of immunogenicity of the vaccine prepared from any type of a strain can protect against other strains of the same type of virus. However, the changes in the antigenic structure which occur gradually during the course of natural variability will not, in the future, be covered even by a high degree of immunity to the old strain. Therefore, the conclusion that old strains in the vaccine should be substituted by new ones -- a conclusion which these authors did not support -- is more correct. It should also be noted that the epidemiological efficacy of the Salk vaccine was not determined by him; in the checking experiments of Philip (1954) it was shown to be of little effectiveness.

It is interesting to dwell on the articles of eminent foreign specialists, Hirst (1949), Andrewes (1952) and Francis (1952, 1953), in which the results were presented of the study of problems of anti-influenza inoculation prophylaxis.

All three authors agreed that vaccination is a promising and very important means of controlling influenza. They advise vaccinating in October-November, that is, during the period following which epidemic outbreaks usually occur in latitudes of the temperate climate, and they suggest that new strains isolated in the last epidemic be included in the vaccine. At the same time, they believed that the efficacy of vaccination was still inadequate, and that therefore perfection of the vaccines was required.

Speaking about the conditions of carrying out the vaccination, Andrewes considers it unnecessary if an epidemic outbreak has been noted in the population in a given locality in the preceding year.

The authors note also the complexity of the subcutaneous method of vaccination, which complicates carrying out this measure on broad scales necessary for protecting large masses of the population against infection.

After noting the costliness of the concentrated vaccine, Hirst suggests vaccinating only the main staffs of government and medical workers in a planned order. On this subject, Andrewes believes that as yet only those who can buy the vaccine can be inoculated. In short, the authors agree that at the present time we cannot speak of preventing influenza epidemics among the population masses by means of inoculations.

What solution do these authors suggest?

Hirst, without advancing anything concrete, speaks only of the need for carrying out further investigations which may solve this problem in the future. Francis, describing Salk's work, recommends the vaccine which he suggested. Finally, Andrewes concludes that apparently the most promising method in the solution of the problem is intranasal immunization with live virus. However, Andrewes writes: "we still do not know how to obtain attenuated virus with the correct properties" for accomplishing this purpose.

In his last article, devoted to problems of influenza prophylaxis, Francis (1955) summarized the observations on the use of polyvalent subcutaneous vaccine carried out by the Commission on Influenza in the United States. Below, we are presenting these data, beginning with 1950 (Table 115).

Table 115

Year	Type of virus causing morbidity	No of those vaccinated	No of those who became sick	%	No of controls	No of those who became sick in the control group	%
1950	A_1	670	8	1,2	2 082	78	3.7
1951	A_1	2 596	13	0,5	5 228	105	2.01
1952	B	207	15	7,24	430	83	19.32
1953	A_1	5 904	57	0,95	5 527	316	5,7
1953	A_1	2 616	16	0,61	4 865	135	2,77

The author believes that a vaccine constituted in the proper manner is effective and recommends its wider use in practice. Nevertheless, it cannot be overlooked that the data on which Francis basis his view are not very convincing because of the low morbidity rate among the controls. In this respect the experiment of 1952 constitutes an exception; however, it is based on a small number of observations, which deprives it of the necessary reliability.

As has been noted above, Soviet research workers have also studied the prophylactic effect of subcutaneous inactivated vaccines. Using the method of virus concentration on erythrocytes with subsequent elution in reduced volumes of isotonic buffer solution, Sokolov prepared a formalin-inactivated vaccine and used it for the immunization of

people. According to the data of Solov'yev and Sokolov (1948), the subcutaneous administration of one cubic centimeter of such a vaccine was used for the immunization of 898 persons, who were divided into three age groups: adolescents aged 14-16; young adults, 19-25; and persons over 30. The results of the record of vaccine reactions in those inoculated are presented below (Table 116).

Table 116

Reactions of Subcutaneous Formalinized Vaccine

Age of those inoculated in years	No of those inoculated	General reactions in %			Local reactions in %		
		severe (temperature above 38°)	moderate (temperature 37,5° –38°)	slight (temperature less than 37.5°)	severe	moderate (infiltration over 5 mm)	slight (infiltration less than 5 mm)
14—16	198	0	2,6	2,4	0	62,8	37,2
19—25	116	0	2,5	3,4	0	5,2	49,1
30 and over	486	0	4,7	4,7	0	3,7	45,0

As seen from Table 116, in the majority of those inoculated a considerable reaction was noted at the site of injection of the vaccine, which was expressed in hyperemia, edema and the subjective sensation of pain. In part of the persons inoculated a temperature rise was observed which lasted one or two days. In addition, a rise in antibody titer was found in them, and it was shown that the serological changes were found in 84.5 percent of the 116 examined. The average antibody titer in the blood rose by five times against the A virus, and by more than three times against the B virus (Fig. 259). The rise in antibody titer in the nasal secretion was noted in 36 percent of 68 examined; however, the quantity of antibodies in the nasal contents was eight to 14 times less than that in the blood. The highest figures were found in vaccinated adolescents (Table 117).

The fact deserves attention that repeated vaccination given after two weeks did not lead to any considerable change in the antibody level in the blood.

After vaccination the antibody titer dropped slowly. Thus, while the average titer before vaccination was equal to 46, after injection of the vaccine it rose to 344, and

after six months it was equal to 150 (Fig. 260).

Fig. 259. Serological Changes in Those Inoculated with Formol Vaccine Subcutaneously (Sokolov, 1954).

Table 117

Antibody Titers in the Blood of Those Vaccinated with Concentrated Formol Vaccine

Age of those vaccinated in years	No of those inoculated	Antibody titer increased		Arithmetic mean titer of antibodies			
				A		B	
		absolute number	%	before immunization	after immunization	before immunization	after immunization
14 16	26	25	96	59	390	83	287
19 25	39	31	80	89	252	38	109
30 and over	51	42	82	28	134	31	76

The epidemiological efficacy of the vaccination was recorded by Sokolov among 539 persons vaccinated and 652 controls which latter were given a "vaccine" containing formalinized normal allantoic fluid. The morbidity rate in the first group was 6.4 percent; in the second, 11.9 percent (Sokolov, 1950).

In his monograph Sokolov (1954) summarized the results of subcutaneous vaccination with concentrated formol baccine. The injection of the vaccine had produced a not-

able rise in antibody titer in those inoculated, higher against the A virus than the B virus; it was more pronounced in adolescents than in adults (Fig. 261).

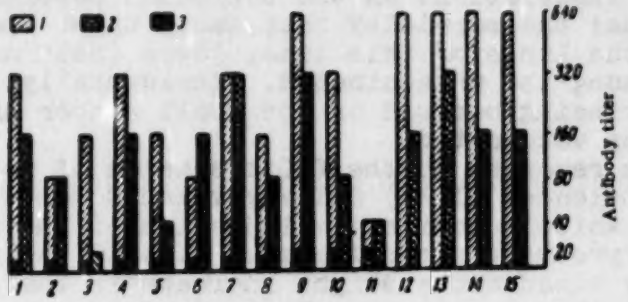

Fig. 260. Antibody Titer Before Inoculations (3), After Two Weeks (1) and Six Months After Inoculations in 15 Vaccinated Persons (Sokolov, 1954).

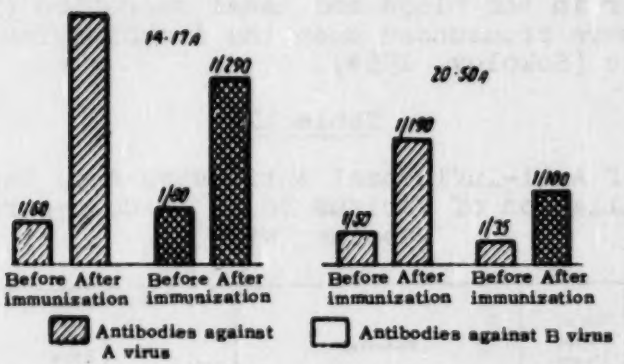

Fig. 261. Rise in Antibody Titer Among Different Age Groups of Those Vaccinated

Key. The figures over the columns represent the arithmetic mean titers of sera (Sokolov, 1954).

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On checking the epidemiological efficacy of the vaccine among 889 persons inoculated it was shown that 1.1 percent became sick; among 320 not vaccinated, 2.5 percent. The inoculations were given a month before the outbreak (1947). In one group it was possible to follow the influence of the inoculations on the influenza morbidity rate for 14 months: the morbidity rate among those inoculated was, after the lapse of this time, lower (half as much) than that among the unvaccinated. Incidentally, these data are not convincing because of the small number of persons in the groups vaccinated.

It is reported at the fifth session of the Academy of Medical Sciences (1948) Solov'yev and Sokolov (1950) reported that while subcutaneous injection of the inactivated vaccine produces a considerable rise in the antibody titer in the blood and a slight increase in the nasal secretion opposite results are obtained after intranasal injection of the same vaccine. By the latter method half the dose of vaccine produced a greater antibody accumulation in the nasal secretion.

Based on this, an attempt was made to apply intranasal immunization of the inactivated vaccine to a mass inoculation influenza prophylaxis experiment. Sokolov, Shimshelevich and Kulikova (1951), using half of the subcutaneous dose, immunized 2854 persons once intranasally. The intranasal instillation of the vaccine caused a rise in antibody titer in the blood and nasal secretion (Table 118), which was more pronounced when the immunization was performed twice (Sokolov, 1954).

Table 118

Formation of Anti-Influenzal Antibodies As a Result of Intranasal Instillation of Various Doses of Concentrated Formol Vaccine Twice

Dose of vaccine in cubic centimeters	No of those inoculated	Material	Arithmetic mean figure of antibody titer		
			before vaccination	after first inoculation	after second inoculation
0.5	22	Serum	10	22	50
		Nasal Secretion	12	36	58
1	15	Serum	5	40	60
		Nasal Secretion	5	30	65

In recording the morbidity rate it was shown that in the inoculated group it amounted to 8.3 percent; in the control group (6800 persons), 17.7 percent (Fig. 262). It should be mentioned also that, in the authors' conclusions, intranasal instillation of the vaccine did not lead to any overt general or local nonspecific reactions.

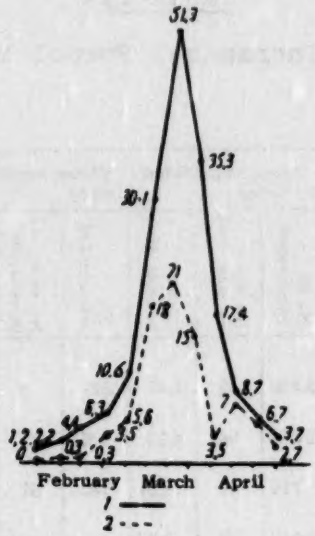

Fig. 262. Influenza Morbidity Rate Among Those Inoculated Intranasally with Concentrated Formol Vaccine and Those Not Inoculated.

1 -- unvaccinated; 2 -- vaccinated (Sokolov, 1954)

Below, the composite Table 119 is presented, according to which it is possible to evaluate the number of observations made and the results obtained.

In the USSR a study was also made of inactivated vaccines with various admixtures. Smorodintsev (1948) considered the addition of the saprophytic acid-resistant bacillus, Mycobacterium butyricum, to the influenzal antigen "exceedingly promising". However, this kind of mixed vaccine (Chalkina, 1949) was not applied practically subsequently. Orlova (1953) used a formalinized influenzal antigen with the addition of calcium phosphate for immunization. This "vaccine with a stimulator," as the author called it, was tried out in large-scale epidemiological experiments.

It was given to people intranasally in a dose of 0.5 cubic centimeter twice using a five- to seven-day interval. In 1950, 3650 persons were vaccinated in this way, but the results could not be recorded because of the absence of an epidemic outbreak. After a year, part of those vaccinated was revaccinated.

Table 119

Efficacy of Intranasal Formol Vaccine Vaccination

Place immunization performed	Those vaccinated twice			Those vaccinated once			Control group			Index of effectiveness
	No of vaccinated	No who became sick	% who became sick	No vaccinated	No who became sick	% who became sick	Total No	No who became sick	% who became sick	
Lodz', boarding schools	2 838	42	1,47	321	8	2,59	363	23	6,33	4,3
Lodz', schools before epidemics	2 074	98	4,72	286	11	3,84	944	101	10,69	2,2
Lodz', schools during epidemics	719	49	6,81	340	37	10,88	305	57	18,68	2,7
Stalingrad, common dwelling places of workers	3 548	79	2,22	—	—	—	1 452	98	6,75	3,04

Records made in the winter of 1952 showed that among 414 persons vaccinated twice the year before an outbreak the morbidity rate amounted to 16.6 percent, while in the control group (306 persons) it was 20.2 percent. Among those revaccinated (160 persons) 5.5 percent became sick; among those not vaccinated (185 persons), 11.3 percent. Here, it should be mentioned that the latter group of persons was inoculated with vaccine containing the A-prime virus, which was not included in the vaccine used earlier.

Extensive epidemiological checking of the given vaccine was accomplished in the winter of 1953/54. According to the data of Samvelova (1954), the results of a single intranasal immunization showed the following figures when the morbidity rate was recorded 10 days after conclusion of the inoculations (Table 120).

Table 120

Group	No. of those inoculated	No. not inoculated	Morbidity rate			
			inoculated		not inoculated	
			absolute number	%	absolute number	%
Workers in industrial enterprises	18 000	8 000	1 056	5,8	482	6
Students	1 207	2 803	67	5,6	192	6,8
School-children	9 211	7 004	986	10,7	664	9,4

At the same time, the vaccine proposed by Orlova was used for the immunization of 25,600 persons by Shapiro, Russina, Chekunova, Nedosekin and Birnbaum (1954). The results of the vaccination are presented in Table 121.

Table 121

Group	No. of those inoculated	No. not inoculated	Morbidity rate in %	
			inoculated	not inoculated
Workers in industrial enterprises	17 941	800	5,8	6
School-children in the higher grades	6 189	4 972	7,2	9
Students in trade schools and industrial training schools	610	400	5,2	6
Medical personnel	110	70	5,4	5,7
Workers in pharmaceutical house	750	800	12	6

As seen from Table 121, no prophylactic effect of the vaccination was found. The authors, analyzing their own data with respect to various institutions observed, came to the paradoxical conclusion that in those groups where the

morbidity rate was low the number of cases among those inoculated was a little more than half of that in the uninoculated; where an outbreak of influenza was noted, that is, where the morbidity rate was increased by four to eight times, the efficacy of vaccination was negligible or nil. In other words, in this case the opposite situation exists compared to what is the rule for inoculation prophylaxis: the reduction in the morbidity rate among those vaccinated is greater the higher the morbidity rate figures.

As a whole, the results of observations made on the study of the vaccine proposed by Orlova do not give us any grounds for appraising it as a good prophylactic measure for influenza.

89. Live Influenza Vaccines

At first, the live influenza virus was used for subcutaneous or intracutaneous vaccination of people (Chenoweth and others, 1936). Francis and Magill (1937) performed immunization experiments on people with an influenza virus culture cultivated on Li and Rivers medium after 160 successive passages made over the course of 11 months. The authors mention that a virus cultivated by this method maintains its ability to cause an experimental infection in white mice and ferrets. The immunization was given subcutaneously and intracutaneously with the live virus. Observations on 23 inoculated persons showed a considerable rise in virus-neutralizing antibodies in 22 of them, which was maintained for five months.

Vaccination with the live virus was given by Chalkina (1938), who injected people subcutaneously with a one-percent suspension of lung tissue of mice infected with influenza in doses of from one to four cubic centimeters. She noted an increase in the virus-neutralizing antibodies in 26 out of 27 persons inoculated, more pronounced than that which was produced by inhalation of the live virus.

In this work it was shown that similar changes in the antibodies of those inoculated may be obtained in persons vaccinated three times with an antigen inactivated by formalin in a dilution of 1:1500.

The latter fact, which has also been noted by a number of other investigators, led to the situation wherein attempts at immunizing people by means of subcutaneous or intracutaneous injection of the live virus were limited to the observations mentioned. The live virus as a vaccine was used afterwards only for introduction into the upper respiratory tract.

The first observations attesting to the possibility of creating an immunity against influenza in man were made

in 1936. They were the result of the work of Smorodintsev and his co-workers who study the pathogenicity of the influenza virus, which had been passaged in experimental animals, for people (Smorodintsev, Drobyshevskaya, Ostrovskaya and Shishkina, 1937).

In 1938, Chalkina's work was published consisting of observations on the immunization of volunteers with the live virus; they received a suspension of the lung tissue of infected ferrets and mice. The following immunization system was used. The first inhalation was a 30-minute inhalation of a suspension in a dilution of 1:200; the second and third were 30-minute inhalations of suspensions in dilutions of 1:50. Forty-three persons were inoculated once according to this system; 59, twice; and 74, three times. As a result of the immunization, no pathological reactions were noted in a single case, and at the same time the serological investigations showed that of the 16 persons vaccinated three times the antibody titer in the blood of 11 increased by 5 to 25 times compared with the original. Even greater serological changes were recorded in those vaccinated who had an initially low level of antibodies. In his conclusions the author mentioned the simplicity of application, the possibility of mass coverage of vaccinated persons and the small expenditure of inoculation material as the advantageous aspects of inhalation immunization.

In 1939, a work was published produced under the direction of Zil'ber in the Moscow Institute imeni Mechnikov by Fal'kovich, Ignatov, Piskunova and Arkhina, who in 1937 also carried out experiments on the intranasal immunization of 38 persons with live virus. A filtrate of lung suspension of infected mice which had been passed through Berkefeld filters served as the antigen. The filtrate was sprayed into the pharynx once or twice in a volume of two cubic centimeters or was instilled into the nose in a volume of one cubic centimeter. As a result of these observations the authors noted the harmlessness of this kind of manipulation and showed that the titer of influenza antibodies rose in the blood of 16 inoculated persons. In addition, an attempt was made at isolating the virus the next day after the immunization from nasopharyngeal washings by means of injecting it into mice using the method of serial passages. The virus was not found in any of those inoculated.

Attempts to isolate the virus from 16 volunteers who in 1944 were given the virus suspension of mouse lungs intranasally were just as unsuccessful (Khayt, Vinogradova and Nartsissov, 1949). In generalizing on the results of these investigations, Zil'ber (1949) pointed out the need of obtaining influenza virus cultures in human tissue ex-

plants which also were to serve as the basis for obtaining effective vaccines. The latter would be affected only if the virus would survive well and multiply well in the mucous membrane of the respiratory tract, producing a response immune reaction in the body.

In 1937, the first experiments were published on the intranasal vaccination of people carried out in Australia by Burnet (1937). As an antigen use was made of a bouillon suspension of chorioallantoic membranes of infected chick embryos which was instilled as drops into the nostrils of six volunteers in a dose of 0.5 cubic centimeter. The author did not observe any pathological symptoms in those inoculated and noted a distinct rise in antibody titer in the blood in two of them. Based on these observations and on the positive results of intranasal immunization of ferrets, he believed that it is possible by this means to create artificially an imitation of a subclinical infectious process which would leave behind it a resistance to the natural disease.

Burnet and Foley (1940), in experiments on volunteers, noted a considerable reduction of thepathogenic and immunogenic properties in laboratory strains of the virus compared with freshly isolated strains. Afterwards, Burnet (1943) immunized 107 persons with a mixture of three Melbourne strains. The first of them had undergone 46 passages through the amniotic cavity of chick embryos; the second, 100; and the third, 40 passages. For the inoculations use was made of allantoic fluid in a dose of 0.25 cubic centimeter, which was introduced into the upper respiratory tract by means of an inhalator. In those inoculated no overt pathological reactions were noted, and in 46 out of the 93 persons examined the antibody titer in the blood rose. Similar results of intranasal immunization were obtained also by Mowson and Swan (1943), who, in addition, noted that the repeated instillation of the vaccine did not reflect essentially in changes in the antibody titer in the blood.

In the work quoted above Burnet reported that intranasal immunization was carried out on several tens of thousands of soldiers among whom there were only solitary complaints of malaise and coryza, which occurred after the introduction of the vaccine. In the entire contingent of those inoculated a prophylactic effect of the vaccine was found in 5,000 persons. Two thousand five-hundred unvaccinated persons served as controls. For the two weeks on record during the epidemic outbreak of influenza A in 1942 the morbidity rate among the former amounted to 4.4 percent; among the latter, 6.63 percent.

Bull and Burnet (1943) conducted the intranasal im-

munization of 23 volunteers by the method described above who were specially selected, because of a low antibody level in the blood, from 100 persons who were examined preliminarily. After the first instillation of the virus an increase in antibody titer in the blood was noted in 21 of the vaccinated persons, and after revaccination, which was done after an interval of from three to six months this rise was established in only two. The investigation of the nasal discharge carried out two days after the inoculation showed the presence of the virus in 14, and after the revaccination in only 3 out of 23 persons examined. The authors noted the absence of any correlation between the detection of the virus in those vaccinated and an increase in the antibody titer in the blood.

A consistent and systematic study of the efficacy of live virus immunization was made after introduction of it into the upper respiratory tract by a group of Leningrad investigators under the direction of Smorodintsev. In generalizing on the accumulated experience in this connection, Smorodintsev and Chalkina (1949, 1952) present summarized data which show that beginning with 1938 extensive comparative observations were made on the influenza morbidity rate in vaccinated and unvaccinated persons. A suspension of lung tissue of infected mice given to people by the inhalation method, described in the work of Chalkina (Table 122), served as the antigen for the immunization.

As seen from Table 122, when the antigen coincides with the virus type producing the disease, the immunized showed half the morbidity rate of the control, unvaccinated persons. In 1940, in Smorodintsev's opinion, the morbidity was produced by the B virus, which was still unknown at that time, and this explains the absence of a positive immunization effect in experiments Nos 4 and 5. The author points out that the essential defect of the inhalation method of immunization is the need for using a quite complex electrical apparatus for spraying and inhaling the antigen as well as the existence of "a small number of reactions, not severe but constant with this type of inoculation". We should like to add on our own that decisive importance should be ascribed, apparently, to the latter circumstance, since in experiment No 4, in which 2694 vaccinated persons were under observations, reactions to the inoculation amounting to a loss of work (4.5 percent) exceeded the influenza morbidity rate figures among the control group of unvaccinated persons (3.1 percent). Smorodintsev and Chalkina (1949) made observations which, in total, included 3456 persons vaccinated with live virus by means of intranasal instillation. A five-percent suspension of lung tissue of infected mice served as the antigen; it was

administered twice or three times at different intervals from two to ten days. The summarized data on this work for the years 1939-1946 are presented below in accordance with the author's material (Table 123).

As seen from Table 123, the reduction in the morbidity rate among those inoculated following intranasal immunization was similar to that noted above for the inhalation method, that is, it was cut in half. An important difference, however, was the absence of pathological reactions in those vaccinated, which is evidence of the preferableness of the intranasal method of immunization.

Smorodintsev and Chalkina (1949), in their further investigations, changed to the cultivation of the virus previously adapted to mice in chick embryos, and for the purpose of activating it used the method of prolonged passages from man to man. They believe that by this means they increased the immunogenic activity of two strains of type A and one strain of type B virus. These strains, when administered to susceptible persons, took hold in the mucous membranes of the upper respiratory tract for two or three days, which was accompanied by an increase in the antibody titer in the blood and in the nasal secretion.

It should be pointed out that in the USSR the first attempt at preparing a live vaccine from virus cultivated in chick embryos was accomplished by Khayt, Vinogradova, Kotina and Edel'man (1942), who in February 1940 immunized 1500 persons intranasally. The antigen was prepared by means of cultivating the A virus on four-day-old embryos and it was tested for virulence by infecting mice. The dose for human vaccination amounted to approximately 1000 minimum lethal doses for mice. This vaccine was instilled intranasally into people twice using an interval of a week. As a result of these inoculations the authors did not note any pathological symptoms after the immunization. Along with this there was no reduction in the morbidity rate among those inoculated either, which was attributed to the different quality of the antigen, which did not coincide with the virus which produced the outbreak of influenza B in March 1940.

Table 122

Results of Active Immunization Against Influenza With Inhalation of Live Type A Virus

No. of experiment	Group make-up: Total	Inoculated	Control	Date of immunization	Reactions to inoculations: % of febrile reactions	% with loss of work	Date of onset of disease	Disease among those vaccinated in the 30 days of observation: absolute No.	%	Disease among those not vaccinated in the 30 days of observation: absolute No.	%	Average duration of disease in days: vaccinated	unvaccinated
				1. In Influenza Produced by A Virus									
1	590	275	315	10/XI. 17/XI. 28/XI 1938	15,8	0,6	26/I 1938	11	4,0	28	8,9	3,1	4,2
2	971	506	465	12/XI. 21/XI 1938	3,0	0	15/II 1939	16	3,1	42	8,5	2,8	3,8
3	2 680	1 296	1 384	1/XII. 8/XII.15/XII 1938	5,2	0	18/II 1939	54	4,1	99	7,1	3,3	4,2
Total...	4 241	2 077	2 164	—	8,0	0,2	—	81	3,9	169	7,8	3,2	4,1
				2. In Influenza of Other Etiology									
4	5 414	2 694	2 720	10/XII. 16/XII. 23/XII 1939	16,1	4,5	26/III 1940	88	3,25	90	3,1	3,8	3,7
5	2 894	1 114	1 780	2/II. 11/II. 20/II 1940	4,3	0,4	16/III 1940	41	3,6	67	3,8	4,1	4,0
Total...	8 308	3 808	4 500	—	10,2	2,5	—	129	3,4	157	3,5	3,8	3,8

Table 123

Results of Active Immunization Against Influenza With Intranasal Instillation of Live Virus

Vaccine	Group Make-up			Immunization date	Reaction to inoculation		Date of onset of disease	Disease among the vaccinated		Disease among the unvaccinated		Average duration of disease in days	
	total	vaccinated	control		% febrile reactions	% with work loss		absolute number	%	absolute number	%	vaccinated	unvaccinated
1. In Influenza Produced by A Virus													
A	2 205	1 084	1 121	20/I, 29/I 1939	0	0	2/II 1939	61	5,6	92	8,2	3,8	4,9
A–B	1 322	940	382	2/II, 4/II, 6/II 1946	0	0	27/I 1946	7	0,74	18	4,7	4,0	4,7
A–B	462	252	210	1/II, 3 II, 5/II 1946	0	0	25/I 1946	16	6,3	26	12,4	2,7	5,6
A–B	448	234	214	8/I, 10/I, 12/I 1946	0	0	25/I 1946	9	3,8	8	3,7	3,5	5,0
Total ...	—	2 510	1 927	—	—	—	—	93	3,7	144	7,4	3,6	5,0
2. In Influenza Produced by B Virus													
A	1 296	946	350	26/II – 7/III 1940	0	0	2/II 1940	93	9,8	33	9,4	4,4	4,0

According to Smorodintsev (1953), Smorodintsev and Chalkina (1953), and Ansheles (1953), live vaccine prepared from the allantoic flora of infected chick embryos was used for the innoculations made in the winter of 1948/49. The first group of students (7,399) was given intranasal immunization with a vaccine containing type A virus; the second group (7,324 persons), with a vaccine containing the B virus; and a third group (6,819 persons), the control group, was not immunized. After two months an epidemic outbreak of influenza began which was produced by the A-prime virus. A part of those inoculated were revaccinated during the development of the outbreak. A record of the morbidity rate showed that in the first group the morbidity rate per thousand persons was equal to 27.5, and in the second and control group, 40.0. In groups of students who were most exposed to the disease and in whom the revaccination was performed the morbidity rate was cut in half.

After 10 months a second increase in the influenza morbidity rate was observed produced by the type B virus. This made it possible to take account of the remote efficacy of immunization with a vaccine containing the B virus. The outbreak of influenza B in 1950 lasted for over two months. The morbidity rate, recorded separately in November and December, showed the following respective indices: among 1,463 persons inoculated with the A vaccine, 10.3 and 7.2 percent; among 1,363 persons innoculated with the B vaccine, 6.8 and 5.9 percent; among 687 control persons, 10.9 and 8.2 percent.

The data presented refer to the vaccination conducted in Leningrad. Similar observations on intranasal vaccination were made in Moscow by Sokolov, Kulikova and Samvelova (1951), who in February-March 1949 immunized 10,096 persons with live vaccine prepared from the allantoic fluid of infected chick embryos. The authors studied the survival of the virus in the nasopharynx of those vaccinated. Of 60 persons examined who were vaccinated once the A virus was isolated in two persons; of 10 persons investigated who were vaccinated three times, it was found in three persons. The virus could be isolated only during the first two days. In all the subjects a considerable antibody rise was noted in the blood and in the nasal secretion. Later, the material of this experiment was made more exact by Sokolov (1954). Of 7,993 persons inoculated with the vaccine containing A virus strains (PR8, M12 and Mak) 6.2 percent became sick, while there was a morbidity rate of 12.2 percent in the control group, which consisted of 10,651 persons. Of 1,881 persons inoculated with the B vaccine (Lee strain), 5.3 percent became sick, and of 4,186 unvaccinated controls in this group 7.5 percent became sick. It

should be noted that vaccination in these experiments was accomplished twice in a dose of 0.5 cubic centimeter introduced into the nostrils by means of a sprayer.

As seen from the data presented, the epidemiological observations made both in Moscow and Leningrad during the epidemic outbreak of influenza A-prime in 1949 showed that the intranasal immunization with a vaccine prepared from type A virus strains exerted an influence in decreasing the morbidity rate among those vaccinated, despite the lack of antigenic correlation.

As has already been mentioned, in the winter of 1950 an outbreak of influenza B occurred. In addition to the data mentioned previously on vaccination accomplished in Leningrad, epidemiological observations made in Moscow (Nikolayev and Samvelova, 1953) and Khar'kov (Zhdanov, 1952; Segal', 1952) the former authors generalized on the results of intranasal immunization of 3,609 persons with a vaccine containing the PR8, Mak (type A) and the YeM-prime (type A-prime) strains as well as the results of immunization of 4,397 persons with type B vaccine prepared from the Lee strain. The immunization was carried out twice with an interval of one day using a dose of 0.5 cubic centimeter by means of spraying.

When these inoculations were delayed they exerted practically no influence on the number of persons who became sick. Among those inoculated with the B vaccine the morbidity rate index was 9.5 percent, and among controls (2,711 persons), 9.4 percent.

A certain reduction in the morbidity rate was shown in the group of those vaccinated with A and A-prime vaccine, which was expressed in indices of 7.4 percent compared with 11.7 percent of those who became ill in the control group, which consisted of 1,202 persons.

In Khar'kov 5,108 persons were inoculated, and better results were obtained with respect to the morbidity rate among 674 immunized children. Immunization with the type B vaccine reduced the morbidity rate by 2.2-2.5 times, and immunization with the A vaccine, by 1.8 times.

As seen from the material presented, after the substitution of preparation of live influenza vaccine from mouse lung tissue by its preparation from the allantoic fluid of chick embryos considerable work was carried out in the USSR in the determination of the suitability of the method of intranasal immunization for the purpose of inoculation prophylaxis of influenza. The results of the observations on the effects of immunization of no less than 38,000 persons showed the harmlessness of this method, its simplicity and its availability for extensive use, but at the same time also its inadequate efficacy. Negative fea-

tures of the liquid vaccines used were shown, which consisted of the lack of standardness of the preparations, their instability even when kept for a short time, the low degree of immunogenicity of the vaccine strains. All this required the organization of considerable experimental investigations directed at improving the quality of the live influenza vaccine.

The principles of obtaining and using the preparations for inoculation prophylaxis of influenza were formulated by Zhdanov and Solov'yev (1952) in the following way:

1. Influenza vaccines should be introduced directly into the upper respiratory tract.

2. In selecting strains for obtaining vaccines the existence of several antigenic strains of the virus should be taken into consideration within limits of the two principal types -- A and B.

3. Immunization should be accomplished with live viruses of adequate immunogenicity but with attenuated pathogenicity for man.

4. The following may serve as the criteria for determining the quality of the vaccine: a) the duration of survival of the virus in the body; b) the absence of pathological signs (fever, rhinitis and tracheitis); c) the accumulation of antibodies in the blood and nasal secretion of those vaccinated. The objective index of the reactivity of those vaccinated is the blood picture and, particularly, the presence of leukopenia. In checking the vaccine this index should be taken into consideration as one of the principal indices of reactivity of the body to the inoculation.

5. The selection of vaccine strains should be accomplished on the basis of the preservation of immunogenic properties characteristic of the freshly-isolated virus, either by means of cultivation in vitro in human tissues or by the combination of these two methods.

6. The proved possibility of maintaining the activity of the influenza virus in a dried state requires utilization of the desiccation method for producing live stable vaccines which are suitable for use for a long time. These principles were developed further in the subsequent works of Zhdanov (1952, 1953, 1954), Solov'yev (1954) and were realized by Fadeyeva (1952), and then by Zakstel'skaya, Ritova (1953, 1954) and Slepushkin (1954).

After strains were obtained which were adapted to human tissue in vitro and it was made clear that these strains survive well and multiply in the nasopharynx of persons who are not immune to influenza (Fadeyeva, 1952), the reactivity of the human body to the administration of them had to be clarified.

This work was carried out by Fadeyeva, Slepushkin,

Ritova and Zakstel'skaya initially in the hospital with the participation of Epshteyn and then under outpatient conditions. It was shown that the instillation of vaccine strains into the nasopharynx produces a reaction in the form of general and local phenomena, which, however, are not very well expressed and disappear rapidly (during the course of one or two days). The general (headache, malaise) and local (rhinitis or pharyngitis) signs are observed only in part of those inoculated and, as a rule, do not lead to any loss of work.

During the clinical observations contact transmission of the vaccine virus was established from vaccinated persons who were in close contact with the persons to whom the disease was transmitted (in the same ward); this was shown by the detection of the virus in the nasopharynx not only in those inoculated but also in the contacts as well as the rise in the titer of specific antibodies against the given virus type in the latter. Only the tissue strains, but not the egg strains, possessed this property (Slepushkin, 1954). However, no further spread of the virus occurred, which was established both by means of special experiments under clinical conditions (Tables 124-125) and by epidemiological observations (Ritova, 1954).

Table 124

Isolation of the Viruses in Those Vaccinated and in Contacts

No. of experiment	Strain variant	Those vaccinated	First contacts	Second contacts
2	Egg	3/6	0/5	0/6
1	Tissue	9/9	4/5	0/20
4	»	6/9	4/9	0/7
5	»	8/8	1/8	0/8
3	»	10/10	—	—

Note. The numerator represents the number of persons in whom the virus was isolated; the denominator, the number of persons participating in the experiment.

Table 125

Isolation of Tissue Strain Variant Following Reinoculation After One and a Half to Two Months

Group for first inoculation	Total examined	Vaccination		Reinoculation after 1½ to 2 months	
		virus isolated	virus not isolated	virus isolated	virus not isolated
Vaccinated	5	4	1	1	4
First contacts	4	2	2	2	2
Second contacts	3	—	3	3	—

Therefore, it has been established that the tissue strains are not very reactive and are harmless to people.

Based on the investigations of Fadeyeva, Zakstel'skaya and Ritova (1953) the methods of obtaining the vaccine strains of influenza viruses were considerably perfected and simplified. The investigations which they conducted showed that the biological properties of the viruses depend on the cells in which the multiplication occurs rather than on the other component parts of the culture medium. Therefore, it has become possible to substitute chick plasma and serum for human plasma and serum, which makes it possible to avoid the advent of alien viruses into the culture with these components.

After studying the process of adaptation of the influenza viruses to human tissues, Fadeyeva, Ritova and Zakstel'skaya showed that this process occurs unequally in different species of viruses (A, A-prime and B), while the rate of adaptation depends on the preceding history of the strains: the old strains, which have been passaged for a long time in chick embryos and mice, adapt slowly to human tissues; in the freshly isolated strains which have been passaged a few times in chick embryos this process occurs rapidly. The A and A-prime viruses were more pliable than the B viruses (Zakstel'skaya and Ritova, 1954; Slepushkin, 1954; Blyumberg, 1954). It was also shown that the establishment of the existence of high and stable virus titers in the tissue cultures signifies the completion of the adaptation process to tissue cultures; in subsequent passages the biological properties of the virus do not change essentially. Finally, it was established that two or three passages in chick embryos do not change the biological pro-

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perties of strains adapted to human tissues.

On the basis of the investigations of Fadeyeva, Ritova and Zakstel'skaya vaccine strains of A-prime viruses (S6, Liga, Botv) and B (Lee, Kri) were obtained in 1952 which survived well and multiplied in the nasopharynx of people (Table 126) and were therefore used for preparing the vaccine.

Table 126

Isolation of the Influenza Virus From Persons Inoculated with Live Influenza Vaccine

No. of person inoculated	Influenza A' virus: recovery of virus from washing according to number of hours after infection of chick embryos 48	72	96	Coefficient of rise in antibody titer	No. of persons inoculated	Influenza B virus: recovery of virus from washing according to no of hours after infection of chick embryos 48	72	96	Coefficient of rise in antibody titer
1	6/6	3/6	2,6	4	14	0/6	3/3	1/3	4
2	3/3	3/3	1/6	64	15	2,6	0/6	0/6	4
3	6/6	3/3	3/3	4	16	0/6	2/6	1/6	4
4	6/6	6/6	6/6	16	17	0/6	0/6	3/6	2
5	3/3	6/6	4/6	8	18	2/6	2/6	1/6	8
6	6/6	4/4	6/6	8	19	0/6	1/6	0/6	2
7	4/4	4/4	5/6	8	20	0/6	1/6	0/6	2
8	4/6	5/6	6/6	32	21	0/6	0/6	2/6	2
9	5/6	4/6	3/6	8	22	3/6	3/6	3/6	8
10	4/4	3/4	3/4	16	23	2/6	1/6	0/6	16
11	6/6	6/6	6,6	4	24	0/6	2/6	0/6	2
12	6/6	6/6	4/5	4	25	1/6	0/6	1/6	4
13	6/6	6/6	6/6	8	26	2/6	1/6	0/6	8

Note. In the denominator, the number of embryos infected; in the numerator, the number of embryos in which the virus was found.

According to the data of Zakstel'skaya (1954), the multiplication of vaccine strains of viruses in the nasopharynx was accompanied by the usual reaction of the body: the appearance and rise in the concentration of antibodies

in the blood and in the nasal secretion (Table 127). A second instillation of the vaccine strain into such persons was not followed by survival of the virus (with rare exceptions). Therefore, an immunity was created in those vaccinated which excluded the possibility of a second vaccinal process (Table 128).

Table 127

Survival of the Virus and Change in the Properties of the Nasal Secretion and Blood in Those Vaccinated Intranasally

Last name of person vaccinated	Virus survival	Reaction of nasal mucosa	Blood antibodies		Neutralizing power of the nasal secretion	
			before vaccination	after vaccination	before vaccination	after vaccination
V–y	+	+	1:40	1:320	100	10 000—100
S–va	+	±	1:10	1:160	10	5 000—500
S–k	+	—	1:20	1:160	10	5 000—500
T–v	+	—	20	1:640	0	10 000—10 000
V–v	+	—	1:10	1:160	10	10 000—10 000
K–v	+	+	1:20	1:160	0	10 000—1 000
D–iy	+	±	1:20	1:40	10	5 000—500
Ki–v	+	—	100	1:40	50	5 000—1 000
S–v	—	—	1:40	1:80	100	1 000—10
Sb–ns	+	+	1:20	1:80	10	1 000—100
S–y	+	—	1:10	1:40	100	50 000—500
Kh–ko	+	—	10	40	not performed	not performed
P–n	+	—	1:20	1:80	» »	» »
No–v	+	+	1:10	1:80	100	100 000
Pa–v	—	—	1:20	1:80	10	10 000
B–n	+	—	1:20	1:80	1 000	10 000
Br–n			1:40	1:80	10	50 000

Zakstel'skaya (1954) studied the activity of vaccinal immunity and showed that in the majority of those vaccinated it is maintained about 10 months; in individual persons it is lost sooner, or, on the other hand, maintained longer.

Continuing their investigations in 1953, Ritova and Zakstel'skaya (1954) showed the possibility of obtaining polyvalent vaccinal strains by means of cultivation of several strains of the same tupe of virus. They obtained a polyvalent A-prime strain ($TChE_5$) by means of the simultaneous cultivation of five A-prime virus strains of different antigenic structures in tissue cultures; polyvalent B strain ($TChE_2$) from two B strain viruses, and then a polyvalent A-

prime strain (TChE7) from seven A-prime virus strains. Each of such polyvalent strains was constituted by means of simultaneous cultivation in human tissues of several viruses of the same type which were different in their antigenic structures.

Table 128

Survival of Virus and Change in Properties of Nasal Secretion and Blood After a Second Vaccination

Last name of vaccinated person	Survival of virus	Reaction of nasal mucosa	Blood antibodies		Neutralizing power of nasal secretion	
			before vaccination	after vaccination	before vaccination	After vaccination
V-y	—	—	160	160	2 000	2 000
S-va	—	—	80	80	1 000	10 000
S-k	—	—	160	160	5 000	5 000
T-v	—	—	640	640	500	10 000
B-v	—	—	40	80	1 000	1 000
K-v	—	—	80	80	500	500
D-y	—	—	40	80	1 000	1 000
Kh-s	—	—	40	80	test not performed	test not performed
S-v	—	—	160	160	500	1 000
Kl-v	—	—	1:40	1:80	1 000	1 000
S-na	—	—	1:80	1:80	1 000	1 000
S-y	—	—	1:40	1:80	500	1 000
P-n	—	—	1:80	1:80	1 000	1 000
N-v	—	—	1:80	1:80	test not performed	test not performed
P-v	—	—	1:40	1:80	» »	» »
V-n	—	—	1:80	1:160	1 000	10000

A study of such mixed cultures showed their polyvalency, and in immunization of animals and vaccination of people they produced an immunity of broader spectrum. At the same time, the virus survival rate was equal to 100 percent or close to it. In the authors' opinion, such strains were very suitable for producing a vaccine; one strain replaced a set of several viruses of the given type (respectively, A, A-prime and B).

Investigations were important which established the number of times vaccination should be performed, the possi-

bility of simultaneous administration of several strains and optimum doses of the vaccine.

The observations of Zakstel'skaya showed that a single administration of the virus is adequate for creating immunity, because when it is administered a second time after two or four weeks it does not survive. Persons in whom, for any reason, the vaccination was unsuccessful constitute an exception in this regard. Therefore, beginning with 1953 the double vaccination was replaced by a single vaccination. As far as the revaccination times are concerned, it was considered expedient to inoculate against influenza once a year. These inoculations are best given in the autumn, in September-November, best during October.

Fadeyeva (1953), Zakstel'skaya and Ritova performed experiments on the study of the possibility of simultaneous administration of the three influenza virus (A, A-prime and B). Ritova (1954) used a mixture of the three viruses in those vaccinated, and then isolated each virus individually from the washings, eliminating the other two viruses by means of neutralization with the respective sera.

These investigations showed that in the non-immune body the simultaneous multiplication of all the three viruses occurs, of the A and A-prime viruses first and then, later, of the B virus. In persons who had influenza B a half-year before, multiplication of the virus in the nasopharynx did not occur.

The optimum doses of the vaccine depend on the totality of the properties of the virus used for immunization. Thus, for example, Fadeyeva and Zakstel'skaya established the dose of vaccine for adults -- 0.3 cubic centimeter of the whole allantoic fluid, that is, 0.1 cubic centimeter of each virus.

Completely different results were obtained in the determination of the vaccine doses for young children who have not had influenza (Ritova and Stefanskaya, 1954). Even in a virus dilution of 10,000 times a multiplication of the virus occurred in the nasopharynx of those vaccinated, although in this dose the virus did not survive in all children. Careful increase in the dosages made it possible to find that a dilution of 1:1000 of the whole allantoic fluid, that is, a dose of 0.003 cubic centimeter, was suitable. In this dose the vaccine survived well and multiplied. It produced a distinct immune reaction and at the same time caused a moderate general and local reaction. Increasing the dose of the virus was associated with an increase in the reactions produced.

The method of vacuum desiccation at low temperature of the vaccine with sucrose-egg-yolk medium was worked out by Sokolov (1952, 1953, 1954). In the allantoic fluid des-

iccated by this method the influenza virus is preserved at a temperature of four degrees up to 21 months without any decrease in virulence for chick embryos. At a temperature of 18° the virulence decreased after five or six months; at a temperature of 37°, after two or three days.

The desiccated vaccine is diluted before immunization to its original volume with physiological solution or distilled water. Smorodintsev (1953, 1954) worked out his own method of desiccating the vaccine and suggested using it in the form of a dry powder, which contained, in addition to the influenza virus, starch used as an inert base. This vaccine is used by means of insufflation of the powder into the nostrils of those being vaccinated.

A comparative study (Sokolova and Gaylonskaya, 1954) causes us to give preference to the first type of vaccine (dry with subsequent solution) over the second (powder-form). Both the biological titers of the vaccine (Table 129) and the better survival of the virus in the nasopharynx of those vaccinated (Table 130) are evidence of this.

Table 129

Titers of Vaccines Used

Series of vaccines	Control number	Strains from which the vaccine was prepared	Form of vaccine	Infectious titers produced by vaccine in embryos
146	104	Commercial type A, A' and B strains from the influenza department of the Institute of Virology imeni Ivanovskiy of the Academy of Medical Sciences USSR (TChE)	Liquid	10^{-8}
163	127			10^{-8}
19	120		Powder	10^{-6}
21	125			10^{-5}
Experimental		Commercial A, A', and B strains of the Dept. of Viruses of Leningrad Institute of Experimental Medicine	Liquid	10^{-8}
191	167			10^{-7}
23	157		Powder	10^{-3}
24				10^{-5}

According to the data of Sokolov (1954), epidemiological observations on the efficacy of the dry vaccine constituted of virus A and A-prime strains were made during

the 1952 outbreak. The vaccine, dissolved in 0.85 percent saline solution, was instilled twice intranasally using a seven-day interval and a dose of 0.4-0.5 cubic centimeter. The record of the morbidity rate in January-April showed that it amounted to 11.5 percent among those vaccinated; 22.1 percent among those not vaccinated. Along with the reduction in the morbidity rate as a result of the inoculations a reduction in the duration of the disease was noted, which was reflected notably in a reduction of the number of days lost from work by those vaccinated.

Table 130

Recovery of Virus From Nasopharyngeal Washings in Days After Vaccination

Vaccine strains	Form of vaccine	No of persons vaccinated	No of those vaccinated from whom the virus was recovered in the washings	Time of isolation of virus in days after vaccination				
				2 days	3 days	4 days	5 days	6 days
Commercial TChE strains	Liquid	19	18	7	15	16	18	4
	Dry	20	16	4	13	12	13	4
Leningrad commercial strains	Liquid	20	16	4	9	9	7	2
	Dry	18	11	6	8	7	2	1
Total		77	61	21	45	44	40	11

Immunization was accomplished twice with the same vaccine in 8,000 persons in Stalingrad by Dubakina, Sutin and Vul'fovich (1953). As a control there were 9,000 unvaccinated persons under observation. The increase in the influenza morbidity rate began at the end of February 1952, one and a half months after conclusion of the inoculations. Among the first group 2.6 percent became sick; among the second, five percent.

Epidemiological observations made on the efficacy of the powdered vaccine proposed by Smorodintsev and Chalkina were made in Leningrad in the winter of 1952/53 by Ansheles, Romanenko, Klyucharev and Danilova (1953). Strains of influenza viruses A, B and A-prime were included in the vaccine. Insufflation of the vaccine intranasally was performed twice in 28,882 persons 10-35 days before the increase in the morbidity rate. Compared with the control group, which included 10,916 persons who had not been given

the vaccine, the authors did not find any reliable reduction in the morbidity rate among those vaccinated, which is explained by the poor quality of the vaccine, particularly by the weakness of the influenzal B-antigen; influenza B was predominant among the diagnoses recorded serologically.

More favorable results were obtained from the testing of other series of this vaccine in Leninakan in the same year (Gukasyan, 1953). Among the 2,136 vaccinated textile workers three percent became sick; among the 2,202 who constituted the controls, unvaccinated, 6.4 percent became sick, that is, the morbidity rate was reduced by 2.1 times among those vaccinated.

In 1952 the first extensive observations were made on the prophylactic effect of the vaccine of a new type, worked out by Zhdanov and Fadeyeva. The vaccine, which was arbitrarily called "a tissue vaccine," contained the vacuum-dried allantoic fluid of chick embryos which had been infected with influenza virus passaged a number of times serially in explants of human embryonic lung tissue.

Fadeyeva and Darienko (1953) inoculated this vaccine into 5,543 persons. The intranasal immunization was accomplished once in a dose of one cubic centimeter of the dry vaccine diluted in boiling water. As a result of their observations the authors concluded that the intranasal instillation of the tissue vaccine produced a general reaction in 9.3 percent of those vaccinated in the form of a headache and a mild malaise, and in 8.3 percent, catarrhal signs of the mucous membrane of the upper respiratory tract. Of the total number of those vaccinated 0.2 percent were sent home from work. The reactions of the body to the intranasal administration of the tissue vaccine were expressed clinically in the next 24-72 hours. The immunological reactions of the body were investigated in 277 persons, among whom a rise in antibody titer of four or more times was found in 197 (71 percent).

Among the groups in whom an increased number of cases of influenza was observed the vaccination reduced the morbidity rate by 2.8 times (Fig. 263).

Similar data have been obtained by Slepushkin (1953): the vaccine proved to be effective in patients from foci of influenza outbreaks (Figs. 264 and 265) and ineffective where the morbidity rate with influenza did not increase and where persons with acute catarrhs of the respiratory tract predominated among the patients. In connection with the predominance of the latter, apparently, all the vaccines did not produce a definite reduction in the morbidity rate at institutions studied by Lozhkina (1953), Ryazanova (1953), Slepushkina (1953) and Movsesyan (1953).

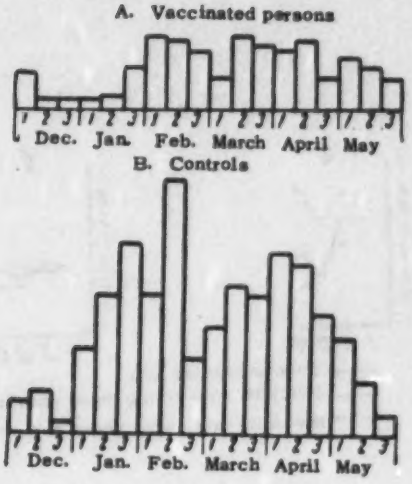

Fig. 263. Analysis of the Total Influenza and Acute Catarrh of the Respiratory Tract Morbidity Rate at a Plant from 1 December 1952 through 1 June 1953 (Fadeyeva and Darienko, 1953).

The results of the epidemiological observations made on the testing of dry live influenza vaccines made in the winter of 1952/53 were summarized by Zhdanov and Nikolayev (1953) and Samvelova (1953). In all, 155,626 persons were vaccinated in Moscow, including 67,631 with a vaccine desiccated according to the Sokolov method (first vaccine), 52,244 persons with the powdered vaccine proposed by Smorodintsev and Chalkina (second vaccine) and 21,381 persons with the tissue vaccine of Zhdanov and Fadeyeva (third vaccine).

There were not many side-reactions produced by any of the vaccines. Local signs in the form of a very transitory catarrh of the nasopharyngeal mucosa were noted in four to nine percent of those vaccinated; insignificant general symptoms in the form of headache and mild malaise, in four to eight percent; temperature rise above 37°, in 0.1-0.6 percent.

As a result of four months of observations it was shown that all the vaccines exerted an influence on the morbidity rate but not to the same degree. In Table 131 the results of the vaccinations are given with vaccines enumer-

ated in the order presented above.

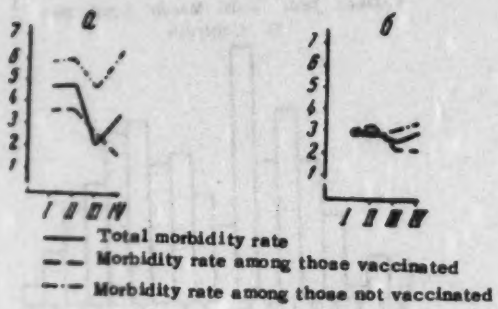

Fig. 264. Dynamics of Morbidity Rate (a) from Influenza and (b) Acute Catarrh of the Respiratory Tract in Persons Inoculated with a Vaccine Prepared by the Institute of Virology (Dose 0.1 cubic centimeter) (Slepushkin, 1954).

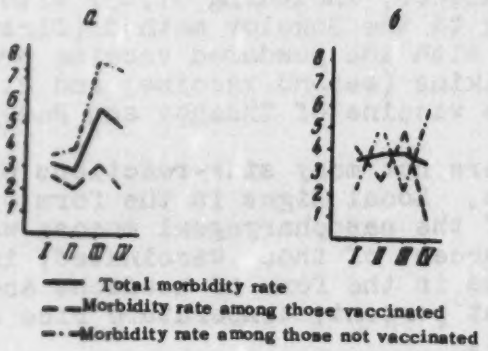

Fig. 265. Dynamics of Morbidity Rate (a) from Influenza and (b) from Acute Catarrh of the Upper Respira-

tory Tract in Persons Inoculated with Vaccine Prepared at the Institute of Virology (Dose 0.5 cubic centimeter) (Slepushkin, 1954).

Table 131

Vaccine	Vaccinated persons		Unvaccinated persons	
	No of persons who became sick per 100	No of days of work lost per 100 persons	No of persons who became sick per 100	No of days of work lost per 100 persons
First	13,9	71,2	16,9	87,8
Second	14,0	58,2	18,3	81,0
Third	10,2	51,7	16,8	87,7

As seen from Table 131, vaccination reduced the morbidity rate and decreased the number of work days lost. The authors point out that during the observation period acute catarrhs of the upper respiratory tract predominated, and virus influenza, according to the data of the serological diagnosis, constituted about a third of all the cases. Such a correction increases the efficacy factor of the vaccination and brings it up to 2.2 to 5. Both in the direct calculation of the morbidity rate among those vaccinated and after the correction for specificity of effect better results were given in these observations by the tissue vaccine (Table 132).

Extensive investigations made with the aim of improving the quality of the vaccines based on factual material accumulated as a result of a study of the problems of variability of the influenza virus and of influenzal immunity, made it possible to develop the practice of intranasal immunization on an even broader scale. This was accomplished in the winter of 1953/54, when the Moscow Sanitary Epidemiological Station administered prophylactic inoculations of the live dry influenza vaccine to 548,344 persons. The summarized data of the epidemiological observations have been given by Samvelova (1954), from whose article we are presenting a composite table characterizing the groups observed and the efficacy of vaccination (Table 133).

Table 132

Influenza Morbidity Rate Among Those Vaccinated and Unvaccinated

Vaccine	No who contracted influenza per 100 vaccinated	No who contracted influenza among 100 unvaccinated	Efficacy factor
Institute imeni Mechnikov	2,6	5,6	2,2
Institute of Experimental Medicine	1,8	6,1	3,4
Institute of Virology (Tissue)	1,0	5,6	5,0
Institute of Virology (with stimulator)	2,0	4,7	2,4

Table 133

Vaccine	Group	No of those vaccinated	No of unvaccinated	No who contracted influenza and catarrhs of the upper respiratory tract 10 days after the inoculation			
				vaccinated		unvaccinated	
				absolute number	%	absolute number	%
1. Tissue	Workers in industrial enterprises	44 464	59 491	5 154	11,6	10 454	17,6
	Students (college level)	3 119	3 343	103	3,3	227	6,8
	School–children	28 775	13 607	2 688	9,3	2 396	17,6
2. Powder	Workers in industrial enterprises	14 153	15 825	2 740	19,3	4 081	25,8
	School–children	12 163	5 626	655	5,4	593	10,5
3. Sucrose–egg–yolk	Workers in industrial enterprises	9 356	8 214	1 214	12,9	1 350	16,4
	School–children	6 029	6 145	642	10,6	880	14,3

The A, B and A-prime viruses were included in the vaccines mentioned. The inoculations were given once, by means of the intranasal instillation of drops or with a sprayer for the dry dissolved vaccines in a dose of one

cubic centimeter and insufflation of the powder vaccine. Samvelova has noted that the reduction in the morbidity rate was different among those vaccinated at various institutions observed. The indices of effectiveness of the vaccination ranged within limits of from zero to a five-fold reduction in the morbidity rate. The tissue vaccine gave the most unitypical results.

Data on intranasal immunization with tissue vaccine for this period are given by Topolyanskaya, Lev, Lurina, Bernshteyn, and Gerasimov (1954). The authors showed that the results of vaccination are more distinctly expressed the greater the morbidity rate figures at the institutions observed. Thus, among 2,957 unvaccinated persons 1,199 became sick, or 40.6 percent; among the 9,344 vaccinated persons at the same institutions, 1,533, or 16.4 percent became sick. In groups being observed in which the morbidity rate was not so high, among the unvaccinated groups it amounted to 20 percent (989 out of 4,944 persons); among the vaccinated in comparable groups the morbidity rate was almost the same, that is, 18.2 percent (977 out of 5,357). The authors concluded that as a general calculation for the vaccinated and control groups the effectiveness of the vaccination was equal to 1.7; in the groups with a higher morbidity rate it reached 3.1. They explain this by the fact that in the groups with the higher morbidity rate influenza was predominant; and in those in which the morbidity rate was not so high, acute catarrhs of the upper respiratory tract. Naturally, in the former case the specific protective effect of the vaccine was expressed more distinctly than in the latter. It should not be overlooked, however, that no diagnostic investigations were made, and this probable explanation remained unconfirmed by factual data.

In the same season vaccination against influenza was accomplished with the tissue vaccine in Leninakan (Gukasyan, 1954). The inoculations were given in December to 3,516 textile workers; 3,640 unvaccinated persons served as controls. An outbreak of influenza produced by the A virus occurred in January-February 1954. Among those vaccinated, 98 persons (2.8 percent) became sick; among the unvaccinated, 307 (8.5 percent). Therefore, the morbidity rate among the vaccinated was reduced by three times compared with the unvaccinated, if we consider the entire total of influenzal diseases during the period of observation (January-May).

In the winter of 1953/54 inoculations of the sucrose-egg-yolk vaccine prepared according to the method of Sokolov were accomplished in Podol'sk and Orekhov-Zuyev. In the first of these cities, according to the data of Balakireva and Kropotova (1954), school children and indust-

rial workers were vaccinated. Among 875 school children in the first three grades vaccinated the morbidity rate amounted to 8.2 percent; among 1,841 vaccinated school children in the fourth grade and higher, 3.5 percent. The morbidity rate among those unvaccinated was divided into internal (in the same classes) and external (other schools) controls. Thus, among the first few grades 14.5 percent of 241 school children became sick (internal control), and of 552 of the external control, 12 percent. Among the higher grades these numbers were, respectively, 9.3 percent out of 395 and five percent out of 2,161.

Industrial workers were vaccinated at two enterprises. At the first of them 3.6 percent became sick among 4,330 vaccinated; at the second, 12 percent among 2,220 vaccinated. Internal control of the morbidity rate record showed the corresponding figures -- 6 and 18 percent. As is seen from these data, the morbidity rate figures, like the efficacy of vaccination, were low. Different results were obtained in Orekhov-Zuyev. According to the data of Sokolov (1954), at this city 9,933 persons were vaccinated once. In comparison with the 5,313 unvaccinated the morbidity was reduced by four to seven times at various institutions. In general, among the former it amounted to 4.3 percent; among the latter, 24.5 percent.

The increase in the number of cases of influenza in the winter of 1953/54 in all the localities where these observations were made was etiologically connected with the A-prime virus. It is important to note that the vaccine included strains of similar but not completely identical antigenic structure.

The investigations of Ritova and Zakstel'skaya (1954), Gutman and Mentkevich (1954) showed that virus strains recovered from patients produce incomplete cross immunity reactions with standard type A-prime virus strains obtained previously -- in 1949 and 1952. Checking of the protective properties of the vaccines used in experiments on immunized mice infected with the freshly isolated strains confirmed these data.

In the winter of 1954/55 observations were made on the efficacy of intranasal vaccination during a rise in influenza B morbidity rate.

Neklyudova and Kovylova (1956) studied the morbidity rate among 47,076 vaccinated persons and 39,606 control persons. The total morbidity rate figures among the first group amounted to four percent; among the second, 10 percent. The authors note that the protective effect of the vaccination was expressed most distinctly among those vaccinated in the rural localities, where the morbidity rate was decreased by three and a half times. The inoculations

were found to be at least effective among workers in tobacco and cement plants, specifically in shops where there was a high degree of dust contamination of the air.

The work of our colleague, Unanov (1956), carried out also in the winter of 1954/55, is very interesting. There were several groups of workers of a cotton combine in Orekhov-Zuyev under our observation. Among them were persons vaccinated in November 1954, persons vaccinated twice -- in 1953 and 1954 -- and persons vaccinated only in the past year, 1953. The results of the morbidity rate records in all of these groups are presented in Table 134 and in Fig. 266, where these figured data may be studied dynamically.

Table 134

Morbidity Rate From Influenza and Acute Catarrh of the Upper Respiratory Tract During the Entire Observation Period (From 1 December 1954 Through 1 April 1955)

Group	No of persons under observation	No who became sick with influenza and acute catarrh of the upper respiratory tract		No of work days lost	Average duration of the disease	No of complications
		Absolute number	%			
Those vaccinated in 1954	6 056	583	9,6	3 125	5,4	6
Those vaccinated twice--in 1953 and 1954	3 931	476	12,2	2 536	5,4	5
Total	9 987	1 059	10,6	5 661	5,3	11
Unvaccinated persons	4 972	1 769	35,4	9 878	5,7	32
Those vaccinated in 1953	1 901	537	28,2	3 066	5,8	4
Total	6 873	2 306	33,6	12 944	5,6	36

Three main conclusions are the results of this work: first, the indisputable fact of a considerable reduction in the morbidity rate among those vaccinated; secondly, the lack of any advantage of a second (after a year) vaccination compared with a single vaccination; third, the lack of any protective effect of vaccination given a year before the given outbreak. The latter permits us to consider that in

this time vaccination immunity to influenza B is lost.

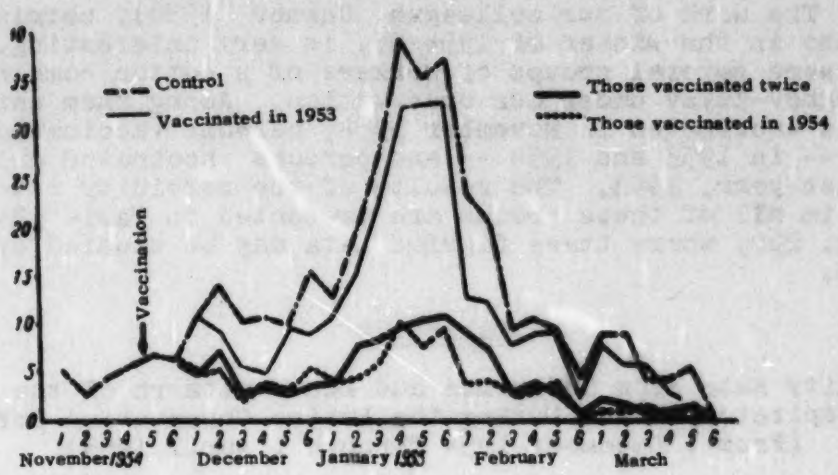

Fig. 266. Influenza Morbidity Rate in Vaccinated and Unvaccinated Groups.

In the observations described a vaccine produced in the Moscow Institute imeni Mechnikov was used.

Therefore, epidemiological observations on the efficacy of intranasal immunization with live dry vaccines, which in recent years have included about 1,000,000 persons, permit us to consider correct the trend chosen by Soviet investigators in developing methods of vaccination prophylaxis of influenza. Official approval of the vaccines used for this purpose has provided the very real possibility of using them in anti-epidemic practice.

Foreign research workers are proceeding along different routes in the solution of this problem. As current data in the literature attest, presented in the chapter devoted to killed vaccines, they are going around in a circle, carrying out considerable work on anti-influenzal immunity. Burnet's experiments on intranasal immunization with the live virus constitute an exception. However, they reflect the theoretical statements of the author, with which we can not agree.

Unfounded fears of a sudden "spontaneous" occurrence of highly pathogenic mutants from the vaccine strains which

would supposedly serve as the cause of artificially produced epidemics devaluate the theoretical aspect of Burnet's investigations. As far as their practical aspect is concerned, we must refer to the words of Andrewes (1952) in this regard, who, after considering intranasal vaccination promising, notes that "unfortunately, we still do not know how to obtain an attenuated virus with the proper characteristics. Burnet's experiments are not promising".

90. Serum Prophylaxis

The first observation on the prophylactic effect of influenzal immune serum in experimental infections in ferrets and mice was made by Laidlaw, Smith, Andrewes and Dunkin (1935). These authors investigated the serum of horses which had been subjected to a prolonged immunization with a filtrate of washings of the respiratory tract of sick ferrets and their lung tissues. They found that this serum possesses the power of neutralizing the influenza virus after a 10-minute contact and of rendering it harmless for intranasal administration into healthy animals. It was shown in experiments on mice that the intraperitoneal injection of immune serum an hour or 24 hours before the infection protect them against the disease. The authors also found that horse influenza antiserum can be concentrated by salting it out with sodium sulfate, whereby the antibodies precipitate with the pseudoglobulin fraction of the serum proteins. The effect of influenzal antiserum has been completely studied by Smorodintsev and Shishkina (1938). They showed that sera of immunized horses, pigs and sheep possess distinct protective properties, containing in one cubic centimeter a quantity of antibodies which neutralizes no less than 1,000 minimum lethal doses for mice. As a result of special investigations it has been established that success in the use of serum for experimental influenzal infection in mice depends to a considerable degree on the method of administration of it. The most effective method has been shown to be intranasal administration or inhalation of drops in a fine spray. Other methods of administering the serum are the following: subcutaneously, intraperitoneally, intravenously. These did not produce such a definite protective effect. The advantages of administering the influenzal antiserum intranasally or by the inhalation method are connected with the selective precipitation of specific antibodies on the surface of the mucous membranes of the upper and lower respiratory tract, where multiplication of the virus occurs.

Stokes and Shaw (1939) came to a similar conclusion concerning the advantages of administering immune serum by

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the intranasal route for the protection of mice against influenzal infection. Afterwards, these investigations were confirmed in the work of Henle, Stokes and Shaw (1941) who studied the effect of immune sera of mice, ferrets, horses, rabbits and man. The latter authors showed that the intranasal route of administration of the serum requires a smaller quantity of it than any other method and that the time of its prophylactic effect on mice is limited to 10 days. Zellat and Henle (1941) confirmed these data and pointed out that serum prophylaxis of experimental influenzal infection is specific only with respect to the sera corresponding to the virus types used for the infection.

The use of intranasal and inhalation administration of horse immune serum for protecting people against influenza was first suggested and carried out by Smorodintsev. In conjunction with Gulamov and Chalkina (1940) he used serum prophylaxis on a group of 500 persons. The serum was administered by the inhalation method, whereby each person was given about two cubic centimeters of the preparation during 15 minutes of inhalation. The serum was inhaled the second time by the same means after 10-15 days. The morbidity rate was recorded from 17 February through 20 March 1939. In the control group, which amounted to 1,825 persons, 150 became sick, or 8.2 percent, whereas in the group given serum prophylaxis eight persons became sick, or 1.6 percent.

Later, Kofman, Kal'yanova and Lyubinskaya (1953) reported that repeated inhalations of horse influenza antiserum given during an outbreak of influenza cause a reduction in the morbidity rate which, however, is not so distinctly expressed as in the experiment presented above. There were three groups of persons under the observation of these authors, of which the first was given immune serum; the second, normal serum; and the third group, was not given anything. As a result, it was shown that during the observation period the morbidity rate among these groups amounted respectively, to 8.4, 11.6, and 15.5 percent. As is seen, the reduction in the morbidity rate was low, particularly compared with the control group of persons which had been given normal horse serum.

Korovin and Dmitrovskaya (1953) made observations on the efficacy of serum prophylaxis in 836 students in March-April 1949. Horse immune serum was given by means of irrigating the nasal cavity by means of a rubber bulb. Each person received a dose of from 1 to 1.5 cubic centimeters three times at intervals of three to four days.

The numerical data concerning the number of cases in the groups given serum prophylaxis and the control group, consisting of 725 persons, are not given by the authors.

They limited themselves to a schematic diagram of the morbidity rate dynamics (Fig. 267) from which it is seen that the morbidity rate was reduced by approximately two to two and a half times.

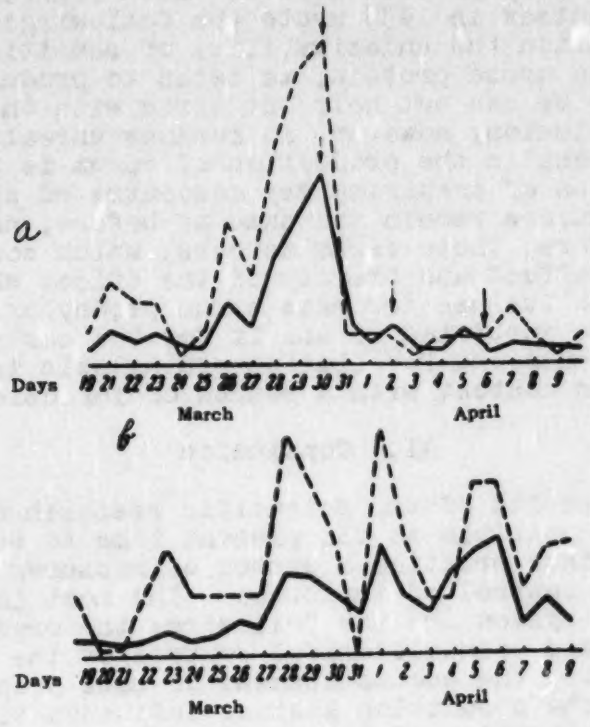

Fig. 267. Influenza Morbidity Rate in Vaccinated and Unvaccinated Groups.

a -- vaccinated; b -- unvaccinated. Solid line -- daily morbidity rate in both groups; broken line -- morbidity rate of school children in the lower grades; the arrows indicate the time the serum was administered.

Everything which has been stated above attests to the possibility of using not only active but also passive immun-

ization with the aim of influenza prophylaxis. The defect of this method is the administration of a foreign protein, horse serum, into the human body; this possesses anaphylactogenic properties. In addition, passive immunization produces only a brief protection against infection, and repeated administrations of the serum are needed to lengthen the prophylactic effect. With regard to the first fact Smorodintsev in 1938 wrote the following: "taking into consideration the undesirability of sensitization of the population to horse protein, we began to produce the serum in steers". We can not help but agree with the correctness of this conclusion; however, it remains unrealized to date. The improvement in the production of serum is proceeding in a direction of preparing dry concentrated preparations, but the producers remain the same as before, namely, horses. Therefore, these serum defects, which consist of the sensitizing effect and brevity of the effect when used once, hardly permit its use for mass serum prophylaxis. We consider it more justified to use it for the early serum therapy of influenza and for limited prophylaxis in focal groups of persons in contact with a source of infection.

91. Conclusion

The results of the scientific research undertaken have made it possible at the present time to work out and incorporate into practice a system of measures for the prophylaxis and control of influenza. The most important elements in the system are the following: the organization of virological and epidemiological control of the influenza morbidity rate, the accomplishment of mass prophylactic vaccination of the population against influenza with live vaccine; the application of influenzal antiserum for treatment and prophylaxis of cases in contacts; the use of antibiotics for preventing complications; the taking of hygenic measures at industrial enterprises; the isolation of patients, and the organization of polyclinic care and home care.

As in the case of other infectious diseases, sanitary education work among the population and work with an active sanitation group are important in the prophylaxis of influenza. The main problem of sanitary propaganda should be the education of the population in information concerning influenza and measures of personal and community prophylaxis (Sigal, 1936; Rossiyskiy, 1949). Taking into consideration the current level of our equipment for the control of influenza the population should be educated not only concerning the value of isolation (even partial) of the patient but also in such measures as the specific pro-

phylaxis and early therapy. Of the other problems which should be clarified in the campaign of sanitation propaganda hygienic problems at factories and under ordinary living conditions should attract attention, as should also propaganda concerning physical culture, athletics and toughening up of the body. Although these prophylactic measures in general apply more to acute catarrhs of the respiratory tract than to influenza, it can still be shown that the healthy, "toughened" organism under favorable living conditions and favorable hygienic conditions at work overcomes any infection better, including influenza. All methods of work with active sanitation groups should be used as much as possible for the purpose of greater success in inculcating hygiene into work and at home: community sanitation authorities, sanitation stations at industries, in schools, state insurance representatives, etc. In this connection, invaluable aid in the prophylaxis of influenza may be given by the trade-union organization and volunteer societies of the Red Cross and Red Crescent.

In conclusion, it should be pointed out that at the present time public health has at its disposal quite effective measures for the prophylaxis of influenza, primarily specific prophylactic measures. While only recently these measures were just objects of scientific investigations, at the present time they have been transferred to the practice of public health organs. Therefore, we may be sure that with the further improvement and incorporation of these measures into practice practically perceptible results of influenza prophylaxis will be achieved to a progressively greater degree in the form of a reduction of the morbidity rate from this infection.

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